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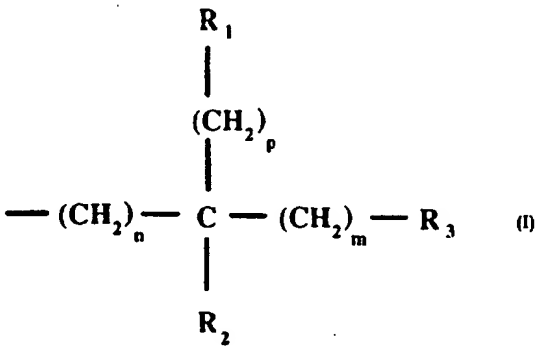
(54) Title: RING-SUBSTITUTED CELL SIGNALING INHIBITORS



(I)

(57) Abstract

Therapeutic compounds with at least one ring-substituted side chain have the formula: CORE MOIETY—$(R)_j$. j is an integer from one to three, the core moiety is non-cyclic or cyclic, and R is selected from the group consisting of hydrogen, halogen, hydroxyl, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups and formula (I), wherein n is an integer from one to twenty and m and p are independently zero or an integer from one to twenty. R_1 is selected from the group consisting of hydrogen, halogen, hydroxide, and substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy, $C_{(2-10)}$ alkenyl, and a ring group having at least one four- to seven-membered ring; R_2 is selected from the group consisting of hydrogen, halogen, hydroxide, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy and $C_{(2-10)}$ alkenyl; and R_3 is selected from the group consisting of hydrogen or a substituted or unsubstituted ring group having at least one four- to seven-membered ring. At least one of R_1 or R_3 is the ring group and a sum of either (n+m) or (n+p), corresponding to a respective R_1 or R_3 ring group is not greater than nineteen. The compounds and pharmaceutical compositions thereof are useful as therapies for diseases advanced via intracellular signaling through specific intracellular signaling pathways by mediating a signaling response to an external stimuli.

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RING-SUBSTITUTED CELL SIGNALING INHIBITORS

Technical Field of the Invention

The invention provides a group of compounds that are effective agents to inhibit specific cellular signaling events often induced by inflammatory stimuli, or to be directly or indirectly antimicrobial to yeast or fungal infections. More specifically, the inventive compounds have at least one ring-substituted chain bonded to a core moiety. The inventive compounds are useful antagonists to control intracellular levels of specific sn-2 unsaturated phosphatidic acids and corresponding phosphatidic acid-derived diacylglycerols, intracellular cell signaling messengers which occur in response to pro-inflammatory proliferative stimuli.

Background of the Invention

Pentoxifylline (1-(5-oxohexyl)-3,7-dimethylxanthine), abbreviated PTX, is a xanthine derivative which has seen widespread medical use for the increase of blood flow. PTX is disclosed in U.S. Patents Nos. 3,422,107 and 3,737,433, both to Mohler et al. Metabolites of PTX were summarized in Davis et al., *Applied Environment Microbiol.* 48:327, 1984. A metabolite of PTX is 1-(5-hydroxyhexyl)-3,7-dimethylxanthine, designated M1. M1 was also disclosed as increasing cerebral blood flow in U.S. Patents Nos. 4,515,795 and 4,576,947 to Hinze et al. Another metabolite, 1-(5-oxohexyl)-3-methylxanthine, designated M6, was disclosed by Bryce et al., *Arzneim.-Forsch./Drug Res.* 39(4):512-517, 1989. In addition, U.S. Patents Nos. 4,833,146 and 5,039,666 to Gebert et al. and Novick, Jr., respectively, disclose use of tertiary alcohol analogs of xanthine for enhancing cerebral blood flow.

PTX and its known metabolites thereof have been shown to have *in vivo* activity in specific biologic systems. U.S. Patent No. 4,636,507 to Kreutzer et al. describes an ability of PTX and M1, to further promote chemotaxis in polymorphonuclear leukocytes responding to a chemotaxis stimulator. In addtion, PTX and related tertiary alcohol substituted xanthines inhibit activity of certain cytokines to affect chemotaxis (U.S. Patents Nos. 4,965,271 and 5,096,906 to Mandell et al.). By administrating PTX and GM-CSF, patients undergoing allogeneic bone marrow transplant exhibited decreased levels of tumor necrosis factor, TNF, (Bianco et al., *Blood* 76: Supplement 1 (522A), 1990). Reduction in assayable levels of TNF was accompanied by a reduction in bone marrow transplant-related complications. However, in normal volunteers, TNF levels were higher among PTX recipients. Therefore, elevated levels of TNF are not the primary cause of such complications.

Further research with PTX, its metabolites and their activity relating to various biologic systems spurred investigations with potential therapeutic agents heretofore unknown.

These agents were identified as potential therapies for treating or preventing disease by inhibiting secondary cellular response to an external or *in situ* primary stimuli. These investigations sought to identify efficacious therapeutic compounds which were safe and effective for human or animal administration and maintain cellular homeostasis in the face of a variety of inflammatory stimuli.

In undertaking these investigations, previously unknown therapeutic compounds were discovered. These novel compounds are discussed herein. These compounds exhibit remarkable characteristics in predictive *in vitro* disease assays, which known compounds do not possess, indicating efficacious therapies for treating or preventing disease using the inventive compounds.

Summary of the Invention

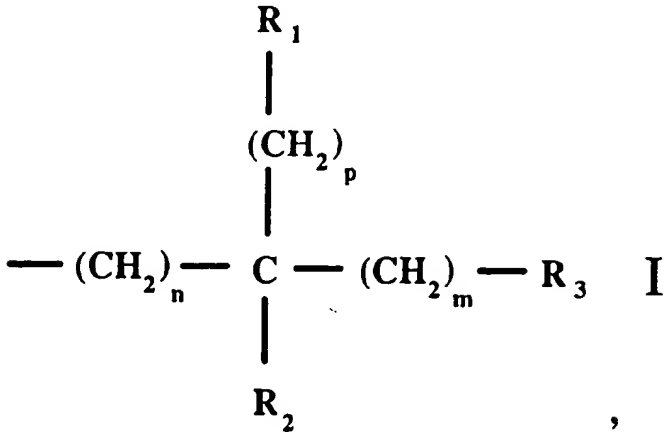
The invention is directed to ring-substituted therapeutic compounds and pharmaceutical compositions and uses thereof. The inventive ring-substituted compounds are useful in a large variety of therapeutic indications for treating or preventing disease. In particular, the inventive compounds and pharmaceutical compositions thereof provide therapy for diseases advanced via intracellular signaling through specific intracellular signaling pathways, specifically the pathways discussed herein, by mediating a signaling response to an external stimuli. Abnormally-induced intracellular signaling is characteristic of diseases treatable using the inventive compounds or pharmaceutical compositions thereof.

The inventive compounds have at least one ring-containing side chain and are preferably cyclic or heterocyclic compounds. The inventive compounds and pharmaceutical compositions thereof have the formula:

$$\text{CORE MOIETY} — (R)_j$$

including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof, wherein j is an integer from one to three, the core moiety is non-cyclic or cyclic and R may be selected from among: hydrogen, halogen (preferably bromine, chlorine, fluorine and iodine), hydroxyl, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups and formula I.

The inventive compounds have at least one R of the following formula I:



wherein n is an integer from one to twenty; m and p are independently zero or an an integer from one to twenty. R_1 is selected from among hydrogen, halogen, hydroxide, and substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy, $C_{(2-10)}$ alkenyl, or a ring group having at least one four- to seven-membered ring; R_2 is selected from among hydrogen, halogen, hydroxide, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy and $C_{(2-10)}$ alkenyl; and R_3 is hydrogen or a substituted or unsubstituted ring group having at least one four- to seven-membered ring. At least one of R_1 or R_3 is the ring group, a sum of either (n + m) or (n + p), corresponding to a respective R_1 or R_3 ring group is not greater than nineteen.

A non-cyclic core moiety may include, but is not limited to, for example, acetamide, amide, amine, amino acid (one or two), carboxide, ester, terminal halogen or hydrogen atom, hydroxide, glutaric acid, glycine derivative, ketone, phosphate, phosphonate, sulfate, sulfonate, sulfone, sulfoxide, simple ionic functional group, thiol, thiolester or the like.

A cyclic core may be at least one five- to seven-member, non-heterocyclic ring or a heterocycle. For example, the core moiety may be selected from the group consisting of substituted or unsubstituted benzene; biphenyl; cyclohexane; cyclohexanedione; cyclopentanedione; napthlalene; phenol; quinone; salicylic acid and derivatives thereof; stilbene, tricyclododecane or the like.

Although other heterocyclic cores are within the scope of the invention, the following representatives are preferred: substituted or unsubstituted barbituric acid; benzamide; lactam; glutarimide; homophthalimide; hydrophthalimide; imidazole; imidazole amide; indomethacin; isocarbostyril; lumazine; N-alkylheterocyclic; N-heterocyclic; pteridine; pthalimide; piperidine; pyridine; pyrimidine; pyrrole amide; quaternized N-heterocyclic;

quinolizinedione; quinazolinone; quinoline; recorsinol; succinimide; theobromine; thymine; triazine; uric acid; uracil; vitamins A, E or K; or xanthine.

Preferably, R is bonded to a nitrogen of the core moiety, if present, most preferably to the nitrogen of a glutarimide, methylthymine, thymine, uracil or xanthine core. In representative, preferred compounds, R having formula I may be bonded to an N_1 nitrogen of glutarimide; N_1 nitrogen of xanthine (and N_3 and N_7 xanthine nitrogens may be independently substituted by a member selected from the group consisting of hydrogen, $C_{(1-6)}$ alkyl, fluoro, chloro and amino); N_3 nitrogen of a thymine or methylthymine; or N_1 nitrogen of uracil. Alternatively, R having formula I may be bonded to N_1 and N_3 xanthine nitrogens and N_7 xanthine nitrogen is substituted by a member selected from the group consisting of hydrogen, methyl, fluoro, chloro and amino;

The invention also provides a pharmaceutical composition. Pharmaceutical compositions of the inventive compounds comprise a pharmaceutical carrier or diluent and some amount of an inventive compound. The nature of the composition and the pharmaceutical carrier or diluent will, of course, depend upon the intended route of administration, for example, parenterally, topically, orally or by inhalation for treatment of a patient with disease symtoms.

The invention includes a method for treating an individual having a variety of diseases. The disease is characterized by or can be treated by inhibiting an immune response or a cellular response to external or *in situ* primary stimuli. Treatment of the disease states involves mediating the cellular response through a specific phospholipid-based second messenger acting adjacent to a cell membrane inner leaflet. The second messenger pathway is activated in response to various noxious or proliferative stimuli, characteristic of disease states treatable using the inventive compounds or pharmaceutical compositions thereof. Biochemistry of this second messenger pathway is described herein. More specifically, the invention includes methods for treating or preventing clinical symptoms of various disease states or reducing toxicity of other treatments by inhibiting cellular signaling through a second messenger pathway involving signaling through phosphatidic acid and through glycan phosphatidylinostinol (Gly PI).

Gly PI consists of a phosphatidylinositol-1-phosphate (PIP) bound through the carbon 6-hydroxyl to a glucosamine residue, which in turn is bound, usually to 2-5 other glycan residues ($1\rightarrow4$ type, linear bonds) containing an additional one to three phosphoethanolamine moieties, the last of which may be bound to an external protein such as Thy-1. Evidence suggests a broad variety of structural variation in the sn-1 and sn-2 positions of the glycerol/lipid moiety of the phosphatidylinositol, as well as fatty acyl addition to the 2-OH group of the inositol. Several functional parameters of structure have been observed, the most remarkable of which point to a minimum presence of at least one myristoyl sidechain in Gly-PI molecules, the

presence of both alkyl (ether) and acyl chains in the sn-1 position, and the presence of palmitate (C16:0) in the 2-OH position of the inositol in protein-binding Gly-PI. Thomas *et al.*, Biochemistry (1991): 29: 5413-5422.

Recent research has demonstrated that 2-OH-acylation of the inositol moiety conveys resistance to hydrolysis with Gly PI-directed phospholipase C (P_iG-PLC, a phosphodiesterase which hydrolyzes Gly PI to glycan inositol phosphate and diacylglycerol) but not to Gly PI-directed phospholipase D (P_iG-PLD, a phosphodiesterase which hydrolyzes Gly PI to glycan inositol + phosphatidic acid).

Research has identified two functions of Gly-PI: 1) external protein binding, the purpose of which may be simple binding to the cell membrane or placement of conformational constraints on the structure of externally bound membrane proteins (*e.g.*, so that a particular portion of the molecule faces an extracellular environment); and 2) signal transduction, including part of the intracellular signal sent by insulin and a detectable portion of the signal transduced by Interleukin-2 (IL-2). We have found that signal transducing Gly-PI in B lymphocytes is hydrolyzed following anti-mu crosslinking, and then resynthesized rapidly. In these systems, two Gly-PI species are synthesized: a) $GlyPI_1$, containing 1-myristoyl 2-palmitoyl, 1-o-tetradecanyl (myristyl) 2-palmitoyl and 1-myristyl 2-myristyl phosphatidylinositol; and b) Gly PI_2, containing 1-myristoyl 2-oleoyl and 1-o-myristyl 2-linoleoyl phosphatidylinositol. Fraction (a) above contains a 1:1 mole content of C22 or C20 acyl groups attached to the inositol phosphate. The Gly-PI_1 fraction, identified by glucosamine labeling followed by mass spectrometry, exhibits a characteristic tripartite peak (glycan-inositol: 2-OH-acyl: phosphatidic acid moieties) and is uniformly inositol 2-OH acylated. Therefore, fraction (a) conveys resistance to P_iG-PLC but not to P_iG-PLD, suggesting that the observed fraction, when hydrolyzed, will generate 1-myristyl and 1-o-myristyl phosphatidic acid species, subsequently observed.

Thus, inventive compounds, useful in treating diseases and reducing toxicity of other disease treatments, would affect cellular signaling through a second messenger pathway by interacting with binding and/or signaling functions of Gly PI.

A disease state or treatment-induced toxicity are selected from the group consisting of: tumor progression involving tumor stimulation of blood supply (angiogenesis) by production of fibroblst growth factor (FGF), vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF); tumor invasion and formation of metastases through adhesion molecule binding, expressed by vascular endothelial cells (VCAM and ICAM); tissue invasion through tumor metalloprotease production such as MMP-9; autoimmune diseases caused by dysregulation of the T cell or B cell immune systems, treatable by suppression of the T cell or B cell responses; acute allergic reactions including, but not limited to, asthma and

chronic inflammatory diseases, mediated by pro-inflammatory cytokines including tumor necrosis factor (TNF) and IL-1, and rheumatoid arthritis, osteoarthritis, multiple sclerosis or insulin dependent diabetes mellitus (IDDM), associated with enhanced localization of inflammatory cells and relase of inflammatory cytokines and metalloproteases; smooth muscle cell, endothelial cell, fibroblast and other cell type proliferation in response to growth factors, such as PDGF-AA, BB, FGF, EGF, etc. (*i.e.*, atherosclerosis, restenosis, stroke, and coronary artery disease); activation of human immunodeficiency virus infection (AIDS and AIDS related complex); HIV-associated dementia; kidney mesangial cell proliferation in response to IL-1, MIP-1α, PDGF or FGF; inflammation; kidney glomerular or tubular toxicity in response to cyclosporin A or amphotericin B treatment; organ toxicity (*e.g.*, gastrointestinal or pulmonary epithelial) in response to a cytotoxic therapy (*e.g.*, cytotoxic drug or radiation); effects of non-alkylating anti-tumor agents; inflammation in response to inflammatory stimuli (*e.g.*, TNF, IL-1 and the like) characterized by production of metalloproteases or allergies due to degranulation of mast cells and basophils in response to IgE or RANTES; bone diseases caused by overproduction of osteoclast-activating factor (OAF) by osteoclasts; CNS diseases resulting from over-stimulation by pro-inflammatory neurotransmitters such as, acetylcholine, serotonin, leuenkephalin or glutamate; acute inflammatory diseases such as septic shock, adult respiratory distress syndrome; multi-organ dysfunction associated with inflammatory cytokine cascade; and combinations thereof.

In a large number of cells, signaling is dependent upon generation of a broad variety of PA species, some of which are generated from lyso-PA by the enzyme lyso-PA acyl transferase and some of which are generated from 2-O-acyl glycan-PI by P_iG-PLD. Generation of each of these PA species (the predominant forms being: 1-acyl and 1-alkyl 2-linoleoyl PA compounds, generated by LPAAT; and 1-myristyl 2-palmitoyl and 1-o-myristyl 2-palmitoyl, generated by P_iG-PLD) serves to effect both proliferative and/or inflammatory signaling in the diseases discussed and cell systems described above.

The inventive compounds are of particular significance for inhibiting IL-2-induced proliferative response. IL-2 signaling inhibition is potentially useful in the treatment of numerous disease states involving T-cell activation and hyperproliferation. Exemplary autoimmune diseases treated by inhibiting IL-2 signaling are lupus, scleroderma, rheumatoid arthritis, multiple sclerosis, glomerula nephritis as well as potential malignancies, including but not limited to, chronic myelogenous leukemia as well as others.

Brief Description of the Drawings

Figure 1 is a dose response curve prepared from results in a murine thymocyte assay, determining inhibitive effects of inventive compounds nos. 2504 and 2507 (see below for chemical name and structure) on thymocyte proliferation.

Figure 2 is a bar graph reporting experimentally calculated IC50 values for inventive compounds nos. 1815, 1816, 2504, 2505, 2507, 2511, 2522, 2526 and 2531.

Figures 3, 4 and 5 are plotted graphs of compound concentrations (μM) and inhibition (as a function of incorporated thymidine, cpm) for compounds nos. 2504, 2522 and 2526, respectively, in a mixed lymphocyte reaction (MLR) assay.

Figure 6 is a bar graph of experimentally calculated IC50 values for inventive compounds tested in the MLR assay of figures 3, 4 and 5.

Figure 7 reports results obtained in a viability assay conducted simultaneously with the MLR assay.

Figure 8 illustrates data collected for inventive compounds nos. 2507, 2511, 2522, 2526, and 2531, illustrating inhibition of human stromal cell proliferation in response to stimulation with platelet derive growth factor (PDGF).

Figure 9 reports data obtained in a proliferation assay, assessing the ability of inventive compound no. 3527 to inhibit proliferation of Balb/3T3 cells stimulated with PDGF.

Figure 10 reports results obtained in a Balb/3T3 viability assay used in conjunction with a proliferation assay.

Figure 11 reports results obtained for inventive compounds nos. 2507, 2511, 2522 and 2526 on inhibition of blast formation from human lymphocytes stimulated by IL-2 or an anti-CD3 antibody.

Figure 12 reports data showing that compound no. 2507 inhibited THP-1 adhesion to IL-1β-stimulated human umbilical vein endothelial cells (HUVEC).

Figure 13 shows that 2507 inhibits TNF secretion in an *ex vivo* human TNF model.

Figure 14 reports inhibitive results obtained for several inventive compounds in a lipo-protein saccharide (LPS)-induced TNF release assay using whole human blood.

Detailed Description of Preferred Embodiments

The invention provides a genus of compounds which can control cellular behavior by a particular phase of a secondary messenger pathway system (Bursten et al., *J. Biol. Chem.* 266:20732, 1991). The second messengers are lipids or phospholipids and use the following abbreviations:

PE = phosphatidyl ethanolamine

LPE = lysophosphoethanolamine

PA = phosphatidic acid

LPA = lysophosphatidic acid

DAG = diacylglycerol

LPLD = lysophospholipase-D

LPAAT = lysophosphatidic acid acyl transferase

PAPH = phosphatidic acid phosphohydrolase

PLA2 = phospholipase A2.

PLD = phospholipase D

PAA = phosphoarachidonic acid

PC = phosphatidyl choline

"remodeled" PA, cyclic pathway = PAA, LPA, PA and DAG intermediates substituted with 1-saturated, 2-linoleoyl or 1,2-dioleoyl, dioleoyl/1,2-sn-dilinoleoyl at the indicated sn-1 and sn-2 positions.

"Classical PI Pathway" = PI, DAG, PA intermediates substituted with 1-stearoyl, 2-arachidonoyl fatty acyl side chains.

"PLD-generated PA" = PE, PC, LPA, PA and DAG intermediates substituted with, *e.g.*, 1,2-sn-dioleoyl-, 1-alkyl, 2-linoleoyl-, and 1-alkyl, 2-docosahexaenoyl-side chains.

Lysophosphatidic acid transferase (LPAAT) effects the synthesis of phosphatidic acid (PA) from lysophosphatidic acid (LPA) by incorporation of an acyl group from acyl CoA. Hydrolysis of the phosphate moiety by PA phosphohydrolase (PAPH) results in the formation of DAG. These aspects of the pathway appear to be activated immediately (within a minute) upon stimulation by a primary stimulus (*e.g.*, a cytokine such as IL-1, IL-2 or TNF) acting at a receptor on a cellular surface. An immediate detectable effect is an elevation of levels of PA and DAG. Administration of the compounds of the invention reverse this elevation.

The compounds and pharmaceutical compositions of the invention include inhibitors of subspecies of LPAAT and PAPH enzymes with substrate specificity for intermediates with 1,2-diunsaturated and 1-alkyl, 2-unsaturated subspecies. One representative example of such an inhibitor (although not within the genus of inventive compounds) is PTX. PTX blocks PAPH in a specific activation pathway that does not involve PI but rather derives from a PA that is largely composed of 1,2-diunsaturated and 1-alkyl, 2-unsaturated subspecies. This was shown, for example, by the demonstration that human mesangial cells stimulated with TNF produce DAG from PI and regenerate PI in the absence and the presence of PTX. In the latter system there is no evidence to suggest that PA or DAG are derived from sources other than PI. It should be emphasized that the compounds of the invention affect that subset of PAPH and

LPAAT that relates to substrates with unsaturated fatty acids other than arachidonate in the sn-2 position, not the housekeeping forms of these enzymes that serve the PI pathway.

Each membrane phospholipid subclass (*e.g.*, PA, PI, PE, PC and PS) reaches a stable content of characteristic fatty acyl side chains due to cyclic remodeling of the plasma membrane as well as turnover for each subclass. PA is often stable, but present in relatively small quantities. PA in resting cells consists mostly of saturated acyl chains, usually consisting of myristate, stearate and palmitate. In resting cells, PC's acyl side chains consist mostly of acyl palmitate in the sn-1 position and oleate in the sn-2 position. PE and PI are predominantly composed of sn-1 stearate and sn-2 arachidonate.

Due to this characteristic content of acyl groups in the sn-1 and sn-2 positions, the origin of any PA species may be deduced from the chemical nature of its acyl groups in the sn-1 and sn-2 positions. For example, if PA is derived from PC through action of the enzyme PLD, the PA will contain the characteristic acyl side chains of PC substrate passed through the second messenger pathway. Further, the origin of any 1,2 sn-substrate species may be differentiated as to its origin. It is important to know whether or not each phospholipid species passes through a PA form prior to hydrolysis to DAG. The lyso-PA that is converted to PA and then to DAG may be shown. The complexities of this second messenger pathway can be sorted by suitable analyses using fatty acyl side chain chemistry (*e.g.*, by thin layer chromatography, gas-liquid chromatography, or high pressure liquid chromatography) of intermediates in cells at various time points after stimulation of the second messenger pathway.

In certain meseachymal cells, such as neutrophils and rat or human mesangial cells, several signaling pathways may be activated in tandem, simultaneously or both. For example, in neutrophils, F-Met-Leu-Phe stimulates formation of PA through the action of PLD, followed in time by formation of DAG through PAPH action. Several minutes later, DAG is generated from PI through the classical phosphoinositide pathway. In many cells, DAG is derived from both PA that is remodeled through a cycle whereby PA is sn-2 hydrolyzed by PLA2, followed by sn-2 transacylation by LPAAT and PA that is generated in a PLD-pathway from either PE or PC or both substrates by PLD.

The present second messenger pathway involves substrates with unsaturated fatty acids in the sn-2 position other than arachidonate and those sub-species of PAPH and LPAAT that are not involved in normal cellular housekeeping functions that are part of the classical PI pathway. The PAPH and LPAAT enzymes involved in this specific second messenger pathway are exquisitely stereo-specific for different acyl side chains and isomeric forms of substrates. Therefore, the inventive compounds may preferably be substantially enantiomerically pure.

PTX (*in vitro*) blocks formation of remodeled PA through the PA/DAG pathway at high PTX concentrations (greater than those that could be achieved in patients without dose-

limiting side effects) by blocking formation of PA subspecies at LPAAT. Even in the presence of PTX, cells continue to form PA through the action of PLD, and DAG is also formed through the action of phospholipase C on PC and PI. The latter pathway are not inhibited by the inventive compounds or PTX. In PTX-treated cells, DAG derived from remodeled and PLA-generated PA is diminished (*e.g.*, 1,2-sn-dioleoyl DAG, 1-alkyl, 2-linoleoyl DAG and 1-alkyl, 2-docosahexaneolyl DAG). Therefore, the inventive compounds and PTX inhibit the formation of only a certain species of PA and DAG by selectively inhibiting a specific second messenger pathway that is only activated in cells by noxious stimuli, but is not used to signal normal cellular housekeeping functions.

Therapeutic Uses of the Inventive Compounds

The specific activation inhibition of the second messenger pathway, as described above and activated primarily by various noxious stimuli, suggests that the inventive compounds are useful in treating a wide variety of clinical indications, mediated at the cellular level by a common mechanism of action. Moreover, *in vitro* and *in vivo* data presented herein provides predictive data that a wide variety of clinical indications, having similar effects on the specific second messenger pathway (activated by noxious stimuli and mediated through, for example, inflammatory cytokines), may be treated by the inventive compounds, which specifically inhibit the pathway. In fact, the mechanism of action for the inventive compounds explains why these compounds have multifarious clinical indications.

Activation of the second messenger pathway is a major mediator of response to noxious stimuli and results in cellular signals that lead to, for example, acute and chronic inflammation, immune response and cancer cell growth. Although the inventive compounds may desirably inhibit other noxious stimuli not discussed, they most effectively mediate the above conditions. Signals mediated by the present second messenger pathway include, for example, those cellular responses of LPS directly; T cell activation by antigen; B cell activation by antigen, cellular responses to IL-1, mediated through the IL-1 Type I receptor (but not the IL-1 Type II receptor), and TNF (Type I receptor), growth stimulated by transformations including, but not limited to, activated oncogenes (*e.g.*, *ras*, *abl*, *her 2-neu* and the like), smooth muscle cell proliferation stimulated by PDGF, b-FGF and IL-1; T cell and B cell growth stimulation by IL-2, IL-4 or IL-7 and IL-4 or IL-6, respectively; and more generally, T cell receptor signaling.

In vitro, the inventive compounds: (1) block IL-1 signal transduction through the Type 1 receptor as shown, for example, by preventing IL-1 and IL-1 plus PDGF (platelet derived growth factor) induction of proliferation of smooth muscle, endothelial and kidney mesengial cells; (2) suppress up-regulation of adhesion molecules as shown, for example, by blocking VCAM in endothelial cells; (3) inhibit TNF, LPS and IL-1 induced metalloproteases

(an inflammation model); (4) block LPS, TNF or IL-1 induced metalloprotease and secondary cytokine production (for prevention and treatment of septic shock); (5) suppress T cell and B cell activation by antigen, for example, IL-2 and IL-4; (6) inhibit mast cell activation by IgE; (7) are cytotoxic for transformed cells and tumor cell lines, yet not for normal cells; and (8) block signaling by IL-2, IL-4, IL-6 and IL-7 on T and B cells.

The foregoing *in vitro* effects give rise to the following *in vivo* biological effects, including, but not limited to: protection and treatment of endotoxic shock and sepsis induced by gram positive or gram negative bacteria; inhibition of tumor cell growth; synergistic immunosuppression, active in autoimmune diseases and in suppressing allograft reactions; and stimulation of hair grow through reversal of an apoptotic process. The inventive compounds are most potent when used to prevent and treat septic shock, treat acute and chronic inflammatory disease, treat or prevent an autoimmune disease and stimulate hair growth (when applied topically).

The inventive compounds also are useful as an adjuvant to inhibit toxic side effects of drugs whose side effects are mediated through the present second messenger pathway.

Metalloproteases mediate tissue damage such as glomerular diseases of the kidney, joint destruction in arthritis, and lung destruction in emphysema, and play a role in tumor metastases. Three examples of metalloproteases include a 92 kD type V gelatinase induced by TNF, IL-1 and PDGF plus bFGF, a 72 kD type IV collagenase that is usually constitutive and induced by TNF or IL-1, and a stromelysin/PUMP-1 induced by TNF and IL-1. The inventive compounds can inhibit TNF or IL-1 induction of the 92 kD type V gelatinase inducable metalloprotease. Moreover, the inventive compounds can reduce PUMP-1 activity induced by 100 U/ml of IL-1. Accordingly, the inventive compounds prevent induction of certain metalloproteases induced by IL-1 or TNF and are not involved with constitutively produced proteases (*e.g.*, 72 kD type IV collagenase) involved in normal tissue remodeling.

The inventive compounds inhibit signal transduction mediated through the Type I IL-1 receptor, and are therefore considered as IL-1 antagonists. A recent review article entitled "The Role of Interleukin-1 in Disease" (Dinarello et al., *N. Engl. J. Med.* 328, 106, Jan. 14, 1993) described the role of IL-1 as "an important rapid and direct determinant of disease... In septic shock, for example, IL-1 acts directly on the blood vessels to induce vasodilatation through the rapid production of platelet activating factor and nitric oxide, whereas in autoimmune disease it acts by stimulating other cells to produce cytokines or enzymes that then act on the target tissue." The article describes a group of diseases that are mediated by IL-1, including sepsis syndrome, rheumatoid arthritis, inflammatory bowel disease, acute and myelogenous leukemia, insulin-dependent diabetes mellitus, atherosclerosis and other diseases including transplant rejection, graft versus host disease (GVHD), psoriasis, asthma, osteoporosis,

periodontal disease, autoimmune thyroiditis, alcoholic hepatitis, premature labor secondary to uterine infection and even sleep disorders. Since the inventive compounds inhibit cellular signaling through the IL-1 Type I receptor and are IL-1 antagonists, the inventive compounds are useful for treating all of the above-mentioned diseases.

For example, for sepsis syndrome, the mechanism of IL-1-induced shock appears to be the ability of IL-1 to increase the plasma concentrations of small mediator molecules such as platelet activating factor, prostaglandin and nitric oxide. These substances are potent vasodilators and induce shock in laboratory animals. Blocking the action of IL-1 prevents the synthesis and release of these mediators. In animals, a single intravenous injection of IL-1 decreases mean arterial pressure, lowers systemic vascular resistance, and induces leukopenia and thrombocytopenia. In humans, the intravenous administration of IL-1 also rapidly decreases blood pressure and doses of 300 ng or more per kilogram of body weight may cause severe hypotension. The therapeutic advantage of blocking the action of IL-1 resides in preventing its deleterious biological effects without interfering with the production of molecules that have a role in homeostasis. The present inventive compounds address this need, identified by Dinarello et al., by inhibiting cellular signaling only through the IL-1 Type I receptor and not through the IL-1 Type II receptor.

With regard to rheumatoid arthritis, Dinarello and Wolff state: "Interleukin-1 is present in synovial lining and synovial fluid of patients with rheumatoid arthritis, and explants of synovial tissue from such patients produce IL-1 *in vitro*. Intraarticular injections of interleukin-1 induce leukocyte infiltration, cartilage breakdown, and periarticular bone remodeling in animals. In isolated cartilage and bone cells *in vitro*, interleukin-1 triggers the expression of genes for collagenases as well as phospholipases and cyclooxygenase, and blocking its action reduces bacterial-cell-wall-induced arthritis in rats." Therefore, the inventive compounds, as IL-1 antagonists, are useful to treat and prevent rheumatoid arthritis.

With regard to inflammatory bowel disease, ulcerative colitis and Crohn's disease are characterized by infiltrative lesions of the bowel that contain activated neutrophils and macrophages. IL-1 can stimulate production of inflammatory eicosanoids such as prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4) and IL-8, an inflammatory cytokine with neutrophil-chemoattractant and neutrophil-stimulating properties. Tissue concentrations of PGE2 and LTB4 correlate to severity of disease in patients with ulcerative colitis, patients with inflammatory bowel disease having high tissue concentrations of IL-1 and IL-8. Therefore, an IL-1 antagonist, such as the inventive compounds, would be effective to treat inflammatory bowel disease.

With regard to acute and chronic myelogenous leukemia, there is increasing evidence that IL-1 acts as a growth factor for such tumor cells. Therefore, the inventive

compounds should be effective to prevent the growth of worsening of disease for acute and chronic myelogenous leukemias.

Insulin-dependent diabetes mellitus (IDDM) is considered to be an autoimmune disease with destruction of beta cells in the islets of Langerhans, mediated by immunocompetent cells. Islets of animals with spontaneously occurring IDDM (*e.g.*, BB rats or NOD mice) have inflammatory cells that contain IL-1. Therefore, the inventive compounds should be useful for the preventing and treating IDDM.

IL-1 also plays a role in atherosclerosis development. Endothelial cells are a target of IL-1. IL-1 stimulates proliferation of vascular smooth muscle cells. Foam cells, isolated from fatty arterial plaques from hypercholesterolemic rabbits, contain IL-1β and IL-1β messenger RNA. The uptake of peripheral blood monocytes results in initiation of IL-1 production by these cells. IL-1 also stimulates production of PDGF. Taken together, IL-1 plays a part in the development of atherosclerotic lesions. Therefore, an IL-1 antagonist, such as the inventive compounds should be useful in preventing and treating atherosclerosis.

IL-1 activates (through the Type I IL-1 receptor) a lyso-PA acyltransferase (LPAAT) and phosphatidate phosphohydrolase within 5 seconds of cell (for example, human mesangial cells, HMC) exposure to this cytokine. As discussed in detail above, activation of both enzymes results in production of PA species with sn-1 and sn-2 unsaturated acyl groups, with the majority of sn-2 acyl chains being polyunsaturated. Both IL-1 and a product of LPAAT, 1,2-sn-dilinoleoyl PA, activate a signaling pathway involving hydrolysis of PE to PA. This reaction is followed by dephosphorylation of PA to produce both 1,2-sn-diacylglycerol, and 1-o-alkyl, or 1-o-alkenyl,acylglycerol (AAG) species. The inventive compounds exert their activity by inhibiting one or both enzymes at an inner leaflet of the plasma membrane. Therefore, appropriate *in vitro* models for drug activity may measure inhibition of stimulation caused by a proinflammatory cytokine or other inflammatory cellular signal.

The generation of the sn-2 unsaturated PA fraction by LPAAT serves to activate either G-proteins, or acts directly upon PLD through alteration of its lipid microenvironment. Activation of LPAAT and generation of the sn-2-unsaturated PA species is an energy sensitive pathway of PLD. This provides a mechanism for a limited-receptor system to amplify a signal and generate a cellular response by rapid synthesis of small amounts of PA. Uptake of di-unsaturated PA, which is less than about 0.1% of total membrane lipid mass, is sufficient to activate PLD activity. This quantity of PA is similar to that endogeneously synthesized by LPAAT. The PA-stimulated PLD acts upon PE, which should be localized to the inner leaflet of the cell membrane, enriched in PE relative to the outer leaflet. Therefore, the cellular inflammatory response to IL-1 is mediated by the pathway: IL-1R → PA → (PLD) → PE. Whereas a localized tissue response is: lysoPA → PI→ PKC → (PLD) → PC. The PLD species

are likely to be different isozymes. The second messenger pathway whose activation is inhibited by the inventive compounds is not a PI-derived pathway and does not involve PKC in the time courses of inhibition. PKC is acutely activated by PI-derived DAG, but chronic activation (*i.e.*, > 30 minutes) is maintained by PC-derived PA generated by PC-directed PLD. Therefore, the pathway inhibited by the inventive compounds is PE-directed and not PC-directed. Moreover, the PE-directed PLD favors substrates with sn-2 long-chain unsaturation.

DAG and PA are upregulated in oncogenically transformed cells. For example, activating *ras* mutations result in increased generation of DAG upon stimulation with mitogens, although the sources of DAG differ between experimental systems. In nontransformed renal mesangial cells, IL-1β stimulation increased PLA2 and LPAAT activation, resulting in generation of sn-2 unsaturated PA and subsequent hydrolysis to DAG by phosphatidate phosphohydrolase. The *ras* transformation in NIH/3T3 cells upregulates serum-stimulated generation of DAG and PA. Particular species of DAG that is stimulated by serum is dioleoyl and of PA are dilinoleoyl and dioleoyl. This upregulation occurs over 4-12 hours and pretreatment of cells with an inventive compound, or PTX, blocks generation of these phospholipid second messengers. The inhibition occurs either through suppressing the generation of PA *de novo* from lysoPA, or through inhibition of one or both arms of the Lands cycle. The coordinate increase of lysoPA in the setting of diminished PA/DAG production suggests inhibition of transacylation of a precursor lipid. Therefore, the *ras* transformation mediates an upregulation of PA through indirect stimulation of PLA2 and/or LPAAT activity. The inventive compounds inhibit the conversion of the upregulated lysoPA to PA and subsequently block the phenotypic changes induced by PA/DAG in the membrane.

The ability of the inventive compounds to inhibit generation of unsaturated phospholipids is mirrored by the ability of inventive compounds to inhibit proliferation and tumorogenicity of *ras*-transformed cells *in vitro* and *in vivo*. PTX inhibits *ras*-transformed NIH/3T3 cells more than parental cells. This inhibition is reversible and is not associated with significant cytotoxicity.

Excessive or unregulated TNF (tumor necrosis factor) production is implicated in mediating or exacerbating a number of diseases including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfusion injury, graft versus host reaction, allograft rejections, fever, myalgias due to infection such as influenza, cachexia secondary to infection, AIDS or malignancy, AIDS, other viral infections (*e.g.*, CMV, influenza, adenovirus, herpes family), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, or pyresis.

The inventive compounds or pharmaceutically acceptable salts thereof can be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of any disease state in a human or other mammal, which is exacerbated or signaled through the present second messenger cellular phospholipid-based signaling pathway and by excessive or unregulated production of "first messenger" inflammatory cytokines such as TNF or IL-1. With regard to TNF first messenger signaling, there are several disease states in which excessive or unregulated TNF production by monocytes/macrophages is implicated in exacerbating or causing the disease. These include, for example, neurodegenerative diseases such as Alzheimers disease, endotoxemia or toxic shock syndrome (Tracey et al., *Nature* 330:662, 1987 and Hinshaw et al., *Circ. Shock* 30:279, 1990); cachexia (Dezube et al., *Lancet* 355:662, 1990), and adult respiratory distress syndrome (Miller et al., *Lancet* 2(8665):712, 1989). The inventive compounds may be used topically in the treatment of prophylaxis of topical disease states mediated or exacerbated by excessive TNF or IL-1, such as viral infections (herpes or viral conjunctivitis), psoriasis, fungal or yeast infections (ringworm, athletes foot, vaginitis, dandruff, etc.) or other dermatologic hyperproliferative disorders. High TNF levels have been implicated in acute malaria attacks (Grau et al., *N. Engl. J. Med.* 320:1585, 1989), chronic pulmonary inflammatory diseases such as silicosis and asbestosis (Piguet et al., *Nature* 344:245, 1990, and Bissonnette et al., *Inflammation* 13:329, 1989), and reperfusion injury (Vedder et al., *Proc. Natl. Acad. Sci. USA* 87:2643, 1990).

The compounds of the invention can inhibit certain VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor) and PDGF (platelet derived growth factor) effects *in vivo*, such as inhibition of angiogenesis or restenosis. For example, Ferns et al., *Science* 253:1129, 1991, have shown that neointimal smooth muscle chemotaxis and angioplasty are inhibited in rats using a neutralizing antibody to PDGF. Also, Jawien et al., *J. Clin Invest.* 89:507, 1992, have shown that PDGF promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. Inhibition of the PDGF-mediated effects following balloon angioplasty by the inventive compounds is the pharmacological rationale for using the inventive compounds as therapeutic agents to prevent restenosis. The inventive compounds also inhibit atherogenesis because increased levels of PDGF expressed by macrophages are associated with all phases of atherogenesis (Ross et al., *Science* 248:1009, 1990). Further, many human tumors express elevated levels of either PDGF, FGF, receptors for FGF or PDGF, or mutated cellular oncogenes highly homologous to these growth factors or their receptors. For example, such tumor cell lines include sarcoma cell lines (Leveen et al., *Int. J. Cancer* 46:1066, 1990), metastatic melanoma cells (Yamanishi et al., *Cancer Res.* 52:5024, 1992), and glial tumors (Fleming et al., *Cancer Res.* 52:4550, 1992).

The inventive compounds are also useful to raise the seizure threshold, to stabilize synapses against neurotoxins such as strychnine, to potentiate the effect of anti-Parkinson drugs such as L-dopa, to potentiate the effects of soporific compounds, to relieve motion disorders resulting from administration of tranquilizers, and to diminish or prevent neuron overfiring associated with progressive neural death following cerebral vascular events such as stroke. In addition, the compounds of the invention are useful in the treatment of norepinephrine-deficient depression and depressions associated with the release of endogenous glucocorticoids, to prevent toxicity to the central nervous system of dexamethasone or methylprednisolone, and to treat chronic pain without addiction to the drug. Further, the compounds of the invention are useful in the treatment of children with learning and attention deficits and generally improve memory in subjects with organic deficits, including Alzheimer's patients.

In Vitro Assays for Physiologic and Pharmacological Effects of the Inventive Compounds

Various *in vitro* assays can be used to measure effects of the inventive compounds to module immune activity and have antitumor activity using a variety of cellular types. For example, a mixed lymphocyte reaction (MLR) provides a valuable screening tool to determine biological activity of each inventive compound. In the MLR, PBMCs (peripheral blood mononuclear cells) are obtained by drawing whole blood from healthy volunteers in a heparinized container and diluted with an equal volume of hanks balanced salt solution (HBSS). This mixture is layered on a sucrose density gradient, such as a Ficoll-Hypaque® gradient (specific gravity 1.08), and centrifuged at 1000 x g for 25 minutes at room temperature or cooler. PBMC are obtained from a band at a plasma-Ficoll interface, separated and washed at least twice in a saline solution, such as HBSS. Contaminating red cells are lysed, such as by ACK lysis for 10 min at 37 °C, and the PBMCs are washed twice in HBSS. The pellet of purified PBMCs is resuspended in complete medium, such as RPMI 1640 plus 20% human inactivated serum. Proliferative response of PBMC to allogeneic stimulation is determined in a two-way MLR performed in a 96-well microtiter plate. Briefly, approximately 10^5 test purified PBMC cells in 200 µl complete medium are co-cultured with approximately 10^5 autologous (control culture) or allogeneic (stimulated culture) PBMC cells, wherein the allogeneic cells are from HLA disparate individuals. Varying doses of compounds (drug) are added at the time of addition of cells to the microtiter plate. The cultures are incubated for 6 days at 37 °C in a 5% CO_2 atmosphere. At the conclusion of the incubation tritiated thymidine is added (for example, 1 µCi/well of 40 to 60 Ci/mmole) and proliferation determined by liquid scintillation counting.

There are a series of *in vitro* assays that can be used to measure immunosuppressive activity of a particular compound. These assays are a predictive model for treatment or

prevention of autoimmune diseases, such as diabetes, lupus, arthritis, and the like. A first assay measures immunosuppressive activity of a drug at the B cell level. Spleens from adult mice contain immature B cells that express surface IgM. Cross-linking the surface IgM with an anti-mu antibody results in B cell proliferation. Additionally, this activation results in an increased expression of interleukin-4 receptors(IL-4R) on the surface of such cells. IL-4 acts as a growth factor for B cells and will increase the amount of proliferation induced by anti-mu. In the first assay, a mixture of anti-mu and murine IL-4 is added to murine splenocytes to cause their proliferation. Mice spleens are obtained from adult mice and a single cell suspension is prepared in RPMI 1640 medium supplemented with 10% FCS. Cells (200,000) are plated into flat-bottomed wells and pre-incubated for 1-2 hrs with various concentrations of drug or PBS if it is a control well. A mixture of anti-mu and murine is added to the wells at a final concentration of 5 μg/ml anti-mu and 12.5 ng/ml IL-4 and plates are incubated for three days. Proliferation is determined on the third day with a pulse of tritiated thymidine. The IC50 concentration of a particular drug is the concentration of drug that results in a 50% inhibition of the proliferation obtained from the positive control.

A second immune suppression assay measures a T cell component to the immune reaction. Lymph nodes contain a mixture of cells including T cells, B cells and macrophages. Although the proliferating cells in this assay are T cells, the response is also dependent upon an antigen presenting cell such as a macrophage as well as an elaboration of various immunoregulatory cytokines. Murine T cells will proliferate *in vitro* in response to a soluble protein antigen if they are first primed with the antigen *in vivo*. *In vivo* priming involves emulsifying the antigen (chicken ovalbumin or OVA) in complete Freunds adjuvant and injecting 50 μg of OVA into both hind footpads of adult Balb/c mice. Fourteen days later the draining lymph nodes (popliteal) are removed and a single cell suspension is prepared in RPMI 1640 supplemented with 10% fetal calf serum. The lymph node cells (200,000) are plated into flat-bottom wells and OVA (200 μg/ml) and/or drug is added to appropriate wells and incubated for 5 days. Proliferation is determined and IC50's calculated as above.

A third assay measures an ability of an inventive compound to inhibit IL-2-induced proliferation of murine thymocytes. Thymus glands are obtained from 4-6 week old mice and plated as a single cell suspension into flat bottomed wells in RPMI 1640 medium supplemented with 10% fetal calf serum. The inventive compounds are added to appropriate wells and the cells are incubated for 1-2 hrs. Concanavilin A (ConA, 0.25 μg/ml) and IL-1 (20 ng/ml) are added and the plates are incubated for 4 days. Cell proliferation is determined as above. There are also variations for this assay that follow the same basic stimulation and measure inhibition of proliferation format. For example, splenocytes can be used instead of thymocytes to measure more of a B cell response than a T cell response (*e.g.*, thymocytes) and

stimulated by an anti-mu antibody (40 μg/ml), IL-4 or PMA (2.5 nM). Similarly, human lymphocytes can be used from normal human volunteers and stimulated with human IL-2 (100 U/ml, Genzyme) and/or anti-CD3 antibody (2.5 μg/ml, Boehinger Mannheim).

Each inventive compound is investigated for cytotoxicity to determine appropriate doses for biological activity assays and to prevent cytotoxic reactions in *in vitro* assays when characterizing activity. Cells (*e.g.*, NIH-3T3, *Ras* transformed 3T3 cells, malignant melanoma LD2 cells, etc.) are added to microtiter plates and drug is added about two days after plating. Cell viability is determined using a fluorescent viability stain (*e.g.*, 2',7'-bis-(2-carboroxyethyl)-5-(and -6)- carboxyfluorescein acetoxymethyl ester, BCECF excitation 488 nm and emission 525 nm) 24, 48 or 72 hours after addition of the drug.

Another assay for measuring activity of the inventive compounds involves determining PDGF (platelet derived growth factor) proliferative response using human-derived stromal cells. Human stromal cells are plated (*e.g.*, about 2000 cells per well) in defined media (*e.g.*, 69% McCoy's, 12.5% fetal calf serum, 12.5% horse serum, 1% antibiotics, 1% glutamine, 1% vitamin supplement, 0.8% essential amino acids, 1% sodium pyruvate, 1% sodium bicarbonate, 0.4% non-essential amino acids and 0.36% hydrocortisone). Two to three days later, the stromal cells are starved in serum-free media. Twenty four hours later, the cells are treated with a stimulating agent, such as PDGF-AA, PDGF-BB or basic FGF (fibroblast growth factor) with or without IL-1α or TNF, and tritiated thymidine. Cell proliferation is determined by liquid scintillation counting.

One *in vitro* assay measures inhibition of the relevant enzymes lysophosphatidic acid acyltransferase (LPAAT) and phosphatidic acid phosphoryl hydrolase (PAPH). The assay involves incubating of target cells with a primary stimulus (*e.g.*, a variety of cytokines, growth factors, oncogene products, putative therapeutic agents, irradiation, viral infection, toxins, bacterial infection and the products thereof, and any stimulus which, if not counteracted, has a deleterious effect on the target cell) in the presence or absence of an inventive compound at varying dosage levels. Target cells include, for example, subcellular entities, such as, microsomes derived from mesenchymal and/or ectodermal cells, particularly microsomes from marrow stromal cells or human or rat mesangial cells; microsomes or synaptosomes derived from bovine brain; plasma membrane-enriched microsomes, plasma membranes derived as described in Bursten et al. (*J. Biol. Chem.* 226:20732-20743, 1991), or detergent-solubilized microsomes; synaptosomes, and membranes or other cell preparations solubilized using, for example, NP-40, Miranal, SDS or other neutral detergents; and detergent-solubilized, recombinant, or further purified preparations of cell proteins, including the proteins LPAAT and/or PAPH. After incubation for short periods of time, cell lipids are extracted and assayed by thin layer chromatography according to standard procedures. Briefly, lipids are extracted using,

for example, chloroform:methanol 2:1 (v/v), and the extracts are then subjected to HPLC as described in Bursten and Harris, *Biochemistry* 30:6195-6203, 1991. A Rainin® mu-Porasil column is used with a 3:4 hexane:propanol organic carrier and a 1-10% water gradient during the first 10 minutes of separation. Detection of the peaks in the elution pattern is by absorption in the range of ultraviolet which detects isolated double bonds. The relevant peaks of unsaturated PA and DAG are shown in the elution pattern. It is important to note that the assay method permits discrimination between various forms of PA and DAG so that those relevant to the pathway affected by the (R) or (S) compounds of the invention can be measured directly. Confirmation of the nature of the acyl substituents of these components is accomplished using fast-atom bombardment mass spectroscopy. Thus, the relevant unsaturated (non-arachidonic) PA and DAG subspecies may be detected. The time periods employed are 5-60 seconds after stimulation with the primary stimulus, such as a cytokine. This technique permits assessment of the levels of various lipid components as a function of time.

An *ex vivo* human sepsis model is described in Desch et al., *Lymphokine Res.* 8:141, 1989 and in Ooi et al., *J. Exp. Med.* 174:649, 1991. Briefly, whole blood from humans is incubated with LPS (endotoxin). TNF released from cells (mostly from monocytes) is measured. The inventive compounds lower TNF levels as compared with untreated controls in this model.

Compounds of the Invention

The invention provides for a class of compounds that are effective agents to inhibit specific cellular signaling events. The inventive compounds and inventive pharmaceutical compositions thereof have the formula:

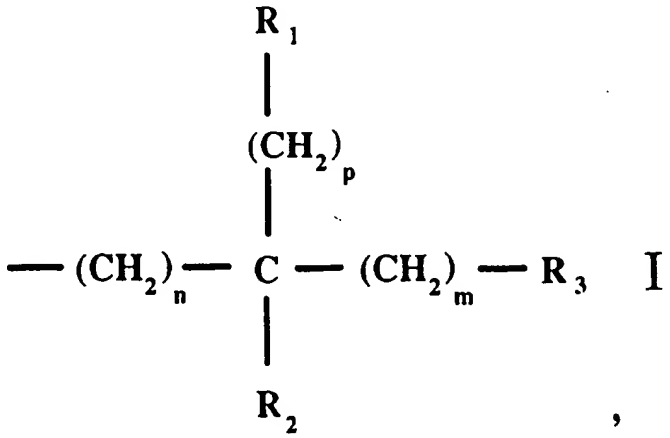
$$\text{CORE MOIETY} — (R)_j$$

including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof, wherein j is an integer from one to three, the core moiety is non-cyclic or cyclic and R may be selected from among: hydrogen, halogen (preferably bromine, chlorine, fluorine and iodine), hydroxyl, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups and formula I.

Preferred R substituents having a structure other than formula I include, but are not limited to, 2-bromopropyl, 4-chloropentyl, cyclohexyl, cyclopentyl, 3-dimethylaminobutyl, ethyl, hexyl, 2-hydroxyethyl, 5-hydroxyhexyl, 3-hydroxy-n-butyl, 3-hydroxypropyl, isobutyl, isopropyl, 2-methoxyethyl, 4-methoxy-n-butyl, methyl, n-butyl, n-propyl, phenyl, t-butyl and

the like. Particularly preferred R having a structure other than formula I are ethyl, methyl, or hydrogen.

The inventive compounds have at least one R of the following formula I:



wherein n is an integer from one to twenty; m and p are independently zero or an an integer from one to twenty. R_1 is selected from among hydrogen, halogen, hydroxide, and substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy, $C_{(2-10)}$ alkenyl, or a ring group having at least one four- to seven-membered ring; R_2 is selected from among hydrogen, halogen, hydroxide, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy and $C_{(2-10)}$ alkenyl; and R_3 is hydrogen or a substituted or unsubstituted ring group having at least one four- to seven-membered ring. At least one of R_1 or R_3 is the ring group, a sum of either (n + m) or (n + p), corresponding to a respective R_1 or R_3 ring group is not greater than nineteen. In the inventive compounds, when the core moiety is xanthine or a substituted derivative thereof, n is not less than two. Optionally, $(CH_2)_n$, $(CH_2)_m$ and/or $(CH_2)_p$ may 1) be substituted by a halogen, hydroxide, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group; 2) have one or two unsaturated bonds (preferably in a *cis* configuration); or 3) be interrupted by at least one oxygen atom.

Preferably, n is an integer from about three to about eighteen, more preferably, an integer from about three to about seven. In especially preferred compounds, p is zero, and R_3 is a substituted or unsubstituted aromatic ring group.

Although other possible substituents are within the scope of the inventive compounds, when R, R_1, and R_2 are substituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl groups; R is substituted cyclic and heterocyclic groups; R_1 and R_2 are substituted $C_{(1-10)}$ alkoxy, and R_1 and R_3 are a substituted ring having at least one four- to seven-membered ring, representative substituents for these groups may be amide, primary, secondary and tertiary amine, $C_{(2-8)}$

alkenyl, $C_{(1-8)}$ alkyl (including, *e.g.*, branched and unbranched alkyl or alkenyl groups), $C_{(1-8)}$ alkoxyalkyl, azide, carbonate, carbonyl, carboxylic acid, cyanide, $C_{(1-8)}$ haloalkyl (including, *e.g.*, mono-, di- and tri-haloalkyl substituents, such as trihalomethyl), isocyanate, isothiocyanate, phosphate, phosphonate, primary, secondary or tertiary alcohol (including, *e.g.*, any one of various diols, methanol, butanol, 1-cyclopentanol, ethanol, 2-ethyl-3-methyl-1-propanol, pentanol, propanol, and methylcyclohexanol), sulfonate, sulfone, sulfoxide, thioamide, thiocarbonate, thioester, thiolester, thiol, thiourea and urea.

Representative R cyclic or heterocyclic groups and R_1 or R_3 ring groups may be, but are not limited to: anthracene, bicyclo[4.4.0]decane, bicyclo[2.2.1]heptane, bicyclo[3.2.0]heptane, bicyclo[4.1.0]heptane, bicylo[2.2.1]hexane, bicyclo[4.3.0]nonane, bicyclo[2.2.2]octane, biphenyl, cyclopentadiene, cyclopentane, cyclobutane, cyclobutene, cycloheptane, cyclohexane, cyclooctane and cyclopropane, 1,2-diphenylethane, fluorene, indene, phenyl, quinone, terphenyl, napthalene, phenanthrene, terphenyl, toluene, xylene, azetidine, benzofuran, benzothiophene, carbazole, furan, glutarimide, indole, isoquinoline, lactam, lactone, oxazole, oxetane, oxirane, phthalimide, piperidine, pyrrolidine, pyran, pyridine, pyrrole, quinoline, tetrahydrofuran, tetrahydropyran, tetrahydrothiophene, thiophene, thymine, derivatives thereof and the like. Due primarily to availability and ease of synthesis, more preferred cyclic groups include less complex ring systems, such as, for example, cyclopentane and cyclohexane, cyclopentadiene, phenyl, indene, toluene, xylene, furan, indole, thymine and xanthine.

A non-cyclic core moiety may include, but is not limited to, for example, acetamide, amide, amine, amino acid (one or two), carboxide, ester, terminal halogen or hydrogen atom, hydroxide, glutaric acid, glycine derivative, ketone, phosphate, phosphonate, sulfate, sulfonate, sulfone, sulfoxide, simple ionic functional group, thiol, thiolester or the like. Exemplary core moiety amino acids may include one or more of the following: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. The non-cyclic core moiety may preferably be an amide, carboxyl ester, carboxide, hydrogen, hydroxide or a dipeptide comprising two amino acids selected from the foregoing exemplary list. A non-cyclic, halogen-core moiety may be, for example, bromine, chlorine, fluorine or iodine.

A cyclic core may be at least one five- to seven-member, non-heterocyclic ring or a heterocycle. The at least one five- to seven-membered cyclic core may preferably have from one to three, five- to six-membered ring structures in a predominantly planar configuration. An exemplary, non-heterocyclic ring core moiety may be selected from the group consisting of substituted or unsubstituted benzene; biphenyl; cyclohexane; cyclohexanedione;

cyclopentanedione; napthlalene; phenol; quinone; salicylic acid and derivatives thereof; stilbene, tricyclododecane or the like.

Although other heterocyclic cores are within the scope of the invention, the following representatives are preferred: substituted or unsubstituted barbituric acid; benzamide; lactam; glutarimide; homophthalimide; hydrophthalimide; imidazole; imidazole amide; indomethacin; isocarbostyril; lumazine; N-alkylheterocyclic; N-heterocyclic; pteridine; pthalimide; piperidine; pyridine; pyrimidine; pyrrole amide; quaternized N-heterocyclic; quinolizinedione; quinazolinone; quinoline; recorsinol; succinimide; theobromine; thymine; triazine; uric acid; uracil; vitamins A, E or K; or xanthine.

Representative substituents for the non-heterocyclic ring and heterocyclic cores may be amide, primary, secondary and tertiary amine, $C_{(2-8)}$ alkenyl, $C_{(1-8)}$ alkyl (including, *e.g.*, branched and unbranched alkyl or alkenyl groups), $C_{(1-8)}$ alkoxyalkyl, azide, carbonate, carbonyl, carboxylic acid, cyanide, $C_{(1-8)}$ haloalkyl (including, *e.g.*, mono-, di- and tri-haloalkyl substituents, such as trihalomethyl), isocyanate, isothiocyanate, phosphate, phosphonate, primary, secondary or tertiary alcohol (including, *e.g.*, any one of various diols, methanol, butanol, 1-cyclopentanol, ethanol, 2-ethyl-3-methyl-1-propanol, pentanol, propanol, and methylcyclohexanol), sulfonate, sulfone, sulfoxide, thioamide, thiocarbonate, thioester, thiolester, thiol, thiourea and urea.

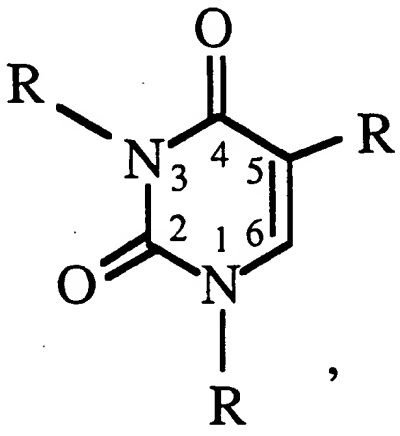
Preferred non-heterocyclic ring cores include substituted or unsubstituted 1,3-cyclohexanedione, 1,3-cyclopentanedione; 1,3-dihydroxynaphthalene; orthophenol.

Preferred heterocyclic cores include substituted or unsubstituted 3,7-dimethylxanthine, glutarimide, 3-methyl-7-pivoloylxanthine, methylthymine, methyluracil, 3-methylxanthine, tetrahydrophthalimide, thymine, uracil and xanthine, most preferably halogen-substituted xanthine. Exemplary preferred cores include: $C_{(1-6)}$ alkyl-substituted thymine; $C_{(1-6)}$ alkyl-substituted uracil; 1,3-dihydroxynapthalene; 3,3-dimethylglutarimide; dihydrothymine; 2,4-dioxohexahydro-1.3.5-tetrazine; hexahydrophthalimide; homophthalimide; 2-hydroxypyridine; β-ionone as vitamin A methylbarbituric acid; 2,6,6-methyl-1-cyclohexene-1-acetaldehyde as vitamin A; methyldihydroxypyrazolopyrimidine, specifically, 1,3-dimethyldihydroxypyrazolo[4,3-d]pyrimidine;1-methyl-5,6-dihydrouracil; 1,7-dimethylxanthine, 3,7-dimethylxanthine; 7-methylhypoxanthine; 1-methyllumazine; 3-methyl-7-methylpivaloylxanthine; methylpyrrolopyrimidine; 1-methylpyrrolo [2,3-d] pyrimidine; 1-methyl-2,4(1H,3H)-quinolizinedione (1-methylbenzoyleneurea); methylthymine; 1-methyluracil; 3-methylxanthine; orotic acid; prostacyclin; 1-pyrrole amides; 2-pyrrole amides; 3-pyrrole amides; quinazolin-4(3H)-one; 1,2,3,4-tetrahydroisoquinolone; tetrahydrophthalimide; sulindac; uracil fused to naphthalene; 5- and/or 6-position substituted uracils (such as, for example, 5-

bromouracil); tetralone to vitamin K; and 8-substituted xanthines (having substituents such as N or S).

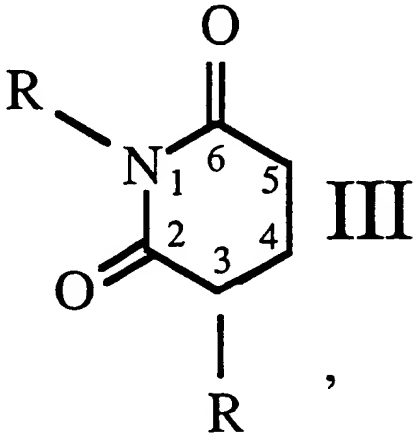
Preferably, R is bonded to a nitrogen of the core moiety, if present, most preferably to the nitrogen of a glutarimide, methylthymine, thymine, uracil or xanthine core. In representative, preferred compounds, R having formula I may be bonded to an N_1 nitrogen of glutarimide; N_1 nitrogen of xanthine (and N_3 and N_7 xanthine nitrogens may be independently substituted by a member selected from the group consisting of hydrogen, $C_{(1-6)}$ alkyl, fluoro, chloro and amino); N_3 nitrogen of methylthymine; or N_1 nitrogen of uracil. Alternatively, R having formula I may be bonded to N_1 and N_3 xanthine nitrogens and N_7 xanthine nitrogen is substituted by a member selected from the group consisting of hydrogen, methyl, fluoro, chloro and amino.

Representative, preferred inventive compounds are compounds of formulas II, III and IV:



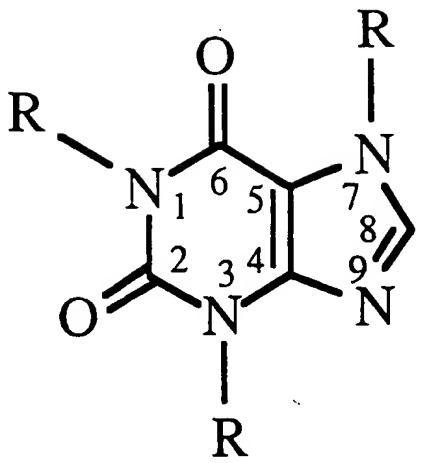
II

,



III

,



IV

wherein R is defined above.

The invention also provides a pharmaceutical composition. Pharmaceutical compositions of the inventive compounds comprise a pharmaceutical carrier or diluent and some amount of an inventive compound. The compound may be present in an amount to effect a physiological response, or it may be present in a lesser amount such that the user will need to take two or more units of the composition to effect the treatment intended. These compositions may be made up as a solid, liquid or in a gaseous form. Or one of these three forms may be transformed to another at the time of being administered such as when a solid is delivered by aerosol means, or when a liquid is delivered as a spray or aerosol.

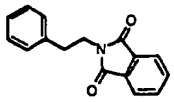
The nature of the composition and the pharmaceutical carrier or diluent will, of course, depend upon the intended route of administration, for example, parenterally, topically, orally or by inhalation for treatment of a patient with disease symtoms. For topical administartion, the pharmaceutical composition will be in the form of a cream, ointment, liniment, lotion, pastes, aerosols and drops suitable for administration to the skin, eye, ear or nose. For parenteral administration, the pharmaceutical composition will be in the form of a steril injectable liquid such as an ampule or an aqueous or non-acqueous liquid suspension. For oral administration, the pharmaceutical composition will be in the form of a tablet, capsule, powder, pellet, atroche, lozenge, syrup, liquid or emulsion.

The invention includes a method for treating an individual having a variety of diseases. The disease is characterized by or can be treated by inhibiting an immune response or a cellular response to external or *in situ* primary stimuli. Treatment of the disease states involves mediating the cellular response through a specific phospholipid-based second messenger acting adjacent to a cell membrane inner leaflet. The second messenger pathway is activated in response to various noxious or proliferative stimuli, characteristic of disease states treatable using the inventive compounds or pharmaceutical compositions thereof. Biochemistry of this

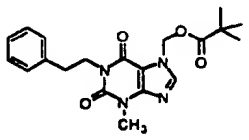
second messenger pathway is described herein. More specifically, the invention includes methods for treating or preventing clinical symptoms of various disease states or reducing toxicity of other treatments by inhibiting cellular signaling through a second messenger pathway involving signaling through phosphatidic acid and through glycan phosphatidylinostinol (Gly PI).

Illustrative, non-limiting, examples of compounds of the invention include the following:

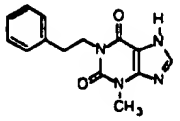
1116 N-(Phenylethyl)phthalimide



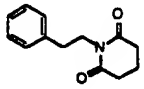
1419 1-(Phenylethyl)-3-methyl-7-methylpivoloylxanthine



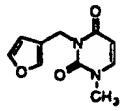
1424 1-(Phenylethyl)-3-methylxanthine



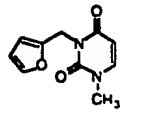
1614 N-(Phenylethyl)-glutarimide



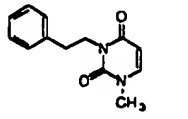
1815 3-(3-Furylmethyl)-1-methyluracil



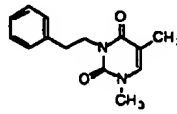
1816 3-(2-Furylmethyl)-1-methyluracil



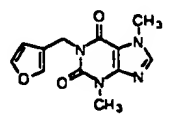
1828 3-(Phenylethyl)-1-methyluracil



1922 3-(Phenylethyl)-1-methylthymine

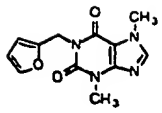


2504 1-(3-Furylmethyl)-3,7-dimethylxanthine

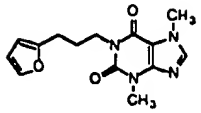


2507 1-(Phenylethyl)-3,7-dimethylxanthine

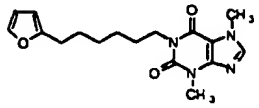
2511 1-(2-Furylmethyl)-3,7-dimethylxanthine



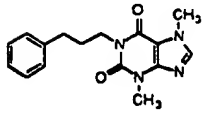
2522 1-[3-(2-Furyl)-propyl)-3,7-dimethylxanthine



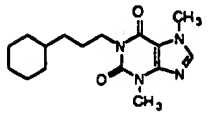
2526 1-[6-(2-Furyl-hexyl)]-3,7-dimethylxanthine



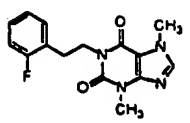
2531 1-(3-Phenylpropyl)-3,7-dimethylxanthine



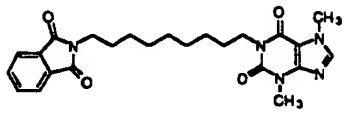
2533 1-(3-Cyclohexylpropyl)-3,7-dimethylxanthine



2585 1-(2-Fluorophenethyl)-3,7-dimethylxanthine



3527 N-(9-Phthalimidononyl)-3,7-dimethylxanthine



Synthesis of the Inventive Compounds

The invention includes a method for preparing the inventive compounds. Exemplary methods for preparing the inventive compounds are discussed below and in the following examples.

In a method according to the invention, a compound containing a cyclic functional group (intended as a ring-substituent in compounds of the invention) is substituted with a carbon chain having the proper length sufficient to later comprise a side arm of the inventive compounds. The cyclic compound undergoes a reaction to produce an anion, which is then subsequently reacted with a substituted carbon chain. The substituted carbon chain has at least one functional group which may be substituted in a displacement reaction by the desired cyclic compound to form a cyclic intermediate, the cyclic intermediate later being attached to a core-containing compound.

Although other reactions are within the scope of the invention, a base may be used to obtain an anion of the cyclic compound. Preferred bases include, but are not limited to, *n*-butyl lithium and lithium diisopropylamine. Preferred substituted carbon chain compounds

Uses of the Invention Compounds and Pharmaceutical Formulations

The inventive compounds provide a method for maintaining homeostasis in cells contacted by primary stimuli by mitigating the effects of these primary stimuli on the secondary signaling pathways invoked within seconds of a primary stimulus. For example, administration of an inventive compound *in vivo* or *ex vivo* provides a method to modify cellular behavior, the method comprising contacting cells (*in vivo* or *ex vivo*), whose behavior is to be modified, with an effective amount of an inventive compound or a pharmaceutical composition thereof. The method is a method to: (1) inhibit proliferation of tumor cells, being; (2) suppress activation of T-cells by antigen or IL-2 stimulation being; (3) suppress activation of monocyte/macrophage cells by endotoxin, TNF, IL-1 or GM-CSF stimulation, being; (4) suppress antibody production of B-cells in response to an antigen, IL-4 or CD40 ligand, being; (5) inhibit the proliferation of smooth muscle cells in response to growth factors capable of stimulating said proliferation, being; (6) lower systemic vascular resistance conferred by endothelial cells, being; (7) lower systemic vascular resistance induced by endothelial cells, being; (8) lower expression of adhesion molecules induced by enhancers thereof, being; (9) suppress the activation of T-cells and macrophages by HIV, being; (10) inhibit the proliferation of kidney mesangial cells in response to stimulation by IL-1 and/or MIP-1α and/or PDGF and/or FGF, being; (11) enhance the resistance of kidney glomerular or tubular cells to cyclosporin A or amphotericin B, being; (12) prevent the release of MIP-1α by IL-1, TNF, or endotoxin stimulated monocytes and macrophages; (13) prevent the release of platelet activating factor by IL-1, TNF, or endotoxin treated megakaryocytes, fibroblastic cells, and macrophages; (14) prevent the down-regulation of receptors for cytokines in TNF-treated hematopoietic progenitor cells, being; (15) suppress the production of metalloproteases in IL-1-stimulated or TNF-stimulated glomerular epithelial cells or synovial cells, being; (16) enhance the resistance of gastrointestinal or pulmonary epithelial cells to cytotoxic drugs or radiation, being; (17) enhance the antitumor effect of a non-alkylating antitumor agent, being; (18) to inhibit the production of osteoclast activating factor in response to IL-1, being; (19) inhibit degranulation in response to IgE, being; (20) enhance the release of adrenergic neural transmitters, dopamine, norepinephrine, or epinephrine, or the neurotransmitter, acetylcholine, being; (21) modulate the post-synaptic "slow current" effects of the adrenergic neurotransmitters dopamine, epinephrine, or norepinephrine, or the neurotransmitter acetylcholine, being; (22) suppress signaling by neurotransmitters including acetyl choline, leuenkephalin and seretonin; or (23) increase seizure theshold.

Indications useful for administering compounds of the invention include, but are not limited to: the presence of a tumor burden, a hormone-related disorder, a neurological disorder, an autoimmune disease, inflammation, restenosis, coronary artery disease, atherosclerosis, hypertension, unwanted immune response (such as allograft reactions), viral

infection, nephritis, mucositis, and various allergic responses. Allergic responses include acute allergic response and thus rhinorrhea, sinus drainage, diffuse tissue edema, and generalized pruritus. As well as the following, other chronic allergic responses include, dizziness, diarrhea, tissue hyperemia, and lacrimal swelling with localized lymphocyte infiltration. Allergic reactions are also associated with leukotriene release and the distal effects thereof, including asthmatic symptoms (*e.g.*, development of airway obstruction, a decrease in FEV1, changes in vital capacity, and extensive mucus production).

Other suitable subjects for the administration of compounds of the invention, include patients: being administered other cytotoxic agents for the treatment of tumors, such as chemotherapeutic agents or irradiation therapy; suffering from neoplasias generally, whether or not otherwise treated including acute and chronic myelogenous leukemia, hairy cell leukemia, lymphomas, megakaryocytic leukemia, and the like; disease states caused by bacterial, fungal, protozoal, or viral infection; exhibiting unwanted smooth muscle cell proliferation in the form of, for example, restenosis, such as patients undergoing cardiac surgery; afflicted with autoimmune diseases, thus requiring deactivation of T and B cells, and having neurological disorders.

The compounds of the invention further are able to decrease enhanced levels of a relevant PA and DAG resulting from stimulation of synaptosomes with acetylcholine and/or epinephrine. This suggests that the effects of the compounds of the invention are to both enhance the release of inhibitory neural transmitters such as dopamine, and to modulate the distal "slow current" effects of such neurotransmitters.

Thus, the drugs of the invention are also useful to raise the seizure threshold, to stabilize synapses against neurotoxins such as strychnine, to potentiate the effect of anti-Parkinson drugs such as L-dopa, to potentiate the effects of soporific compounds, to relieve motion disorders resulting from administration of tranquilizers, and to diminish or prevent neuron overfiring associated with progressive neural death following cerebral vascular events such as stroke. In addition, the compounds of the invention are useful in the treatment of norepinephrine-deficient depression and depressions associated with the release of endogenous glucocorticoids, to prevent the toxicity to the central nervous system of dexamethasone or methylprednisolone, and to treat chronic pain without addiction to the drug. Further, the compounds of the invention are useful in the treatment of children with learning and attention deficits and generally improve memory in subjects with organic deficits, including Alzheimer's patients.

While dosage values will vary, therapeutic efficacy is achieved when the compounds of the invention are administered to a human subject requiring such treatment as an effective oral, parenteral, or intravenous sublethal dose of about 50 mg to about 5000 mg per

day, depending upon the weight of the patient. A particularly preferred regimen for use in treating leukemia is 4-50 mg/kg body weight. It is to be understood, however, that for any particular subject, specific dosage regimens should be adjusted to the individual's need and to the professional judgment of the person administering or supervising the administration of the inventive compounds.

Pharmaceutical Formulations

A suitable formulation will depend on the nature of the disorder to be treated, the nature of the medicament chosen, and the judgment of the attending physician. In general, the inventive compounds are formulated either for injection or oral administration, although other modes of administration such as transmucosal or transdermal routes may be employed. Suitable formulations for these compounds can be found, for example, in *Remington's Pharmaceutical Sciences* (latest edition), Mack Publishing Company, Easton, PA.

The inventive compounds and their pharmaceutically acceptable salts can be employed in a wide variety of pharmaceutical forms. The preparation of a pharmaceutically acceptable salt will be determined by the chemical nature of the compound itself, and can be prepared by conventional techniques readily available. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25 mg to about 1 gram, wherein the amount of inventive compound per dose will vary from about 25 mg to about 1 gram for an adult. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension. Where the inventive composition is in the form of a capsule, any routine encapsulation is suitable, for example, using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule, any pharmaceutical carrier routinely used for preparing dispersions of suspensions may be considered, for example, aqueous gums, celluloses, silicates or oils and are incorporated in a soft gelatin capsule shell. A syrup formulation will generally consist of a suspension or solution of the compound or salt thereof in a liquid carrier (*e.g.*, ethanol, polyethylene glycol, coconut oil, glycerine or water) with a flavor or coloring agent.

The amount of inventive compound required for therapeutic effect on topical administration will, of course, vary with the compound chosen, the nature and severity of the disease and the discretion of the treatment provider. Parenteral includes intravenous, intramuscular, subcutaneous, intranasal, intrarectal, intravaginal or intraperitoneal administration. Appropriate dosage forms for such administration may be prepared by conventional techniques. A typical parenteral composition consists of a solution or suspension of the inventive compound

or a salt thereof in a sterile or non-aqueous carrier, optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil, or sesame oil. The daily dosage for treatment of sepsis or another severe inflammatory condition via parenteral administration is suitable from about 0.001 mg/kg to about 40 mg/kg, preferably from about 0.01 mg/kg to about 20 mg/kg of an inventive compound or a pharmaceutically acceptable salt thereof calculated as the free base.

The inventive compounds may be administered orally. The daily dosage regimen for oral administration is suitably from about 0.1 mg/kg to about 1000 mg/kg per day. For administration the dosage is suitably from about 0.001 mg/kg to about 40 mg/kg of the inventive compound or a pharmaceutically acceptable salt thereof, calculated as the free base. The active ingredient may be administered from 1 to 6 times a day, sufficient to exhibit activity.

The inventive compounds may be administered by inhalation (*e.g.*, intranasal or oral) Appropriate dosage forms include an aerosol or a metered dose inhaler, as prepared by conventional techniques. The daily dosage is suitably from about 0.001 mg/kg to about 40 mg/kg of the inventive compound or a pharmaceutically acceptable salt thereof, calculated as the free base. Typical compounds for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant.

The invention is illustrated by the following examples which should not be regarded as limiting the invention in any way.

Example 1

This example is a method of synthesis for inventive compound no. 1815 (see above for chemical name and structure). Methanesulfonyl chloride (0.49 g, 4.2 mmol) and then triethylamine (0.62 g, 6.2 mmol) were added to a solution of 3-furan methanol (0.40 g, 4.0 mmol) in dichloromethane (10 ml) at 0 °C. After stirring the reaction mixture for 10 minutes at 0 °C, it was allowed to warm to 25 °C and stirred for an additional 2 hours. The reaction was poured into water (10 ml) and separated and washed with dichloromethane (10 ml). The organic portions were combined, dried over magnesium sulfate, and evaporated to give an oil, 3-methanesulfonyl methylfuran, used without further purification.

Sodium hydride (0.10 g, 4.2 mmol) was added to a suspension of 1-methyluracil (0.50 g, 3.96 mmol) in dimethyl sulfoxide (10 ml). The reaction mixture was stirred for 30 minutes at 25 °C, 3-methanesulfonyl methylfuran in dimethyl sulfoxide (5 ml) added and the reaction stirred for an additional 16 hours at 60 °C. The mixture was then poured into water (50 ml) and extracted with two 50 ml aliquots of diethyl ether. The organic portion was dried over magnesium sulfate and the solvent evaporated, leaving a yellow solid. The yellow solid,

purified by chromatography with silica using an ethyl acetate/hexane eluant, yielded 0.45 g of a white solid, compound no. 1815 (55% yield).

Example 2

This example is a method of synthesis for inventive compound no. 2504 (see above for chemical name and structure). Methanesulfonyl chloride (1.92 g, 4.0 ml, 16.8 mmol) and then triethylamine (2.52 g, 25.0 mmol) were added to a solution of 3-furan methanol (1.50 g, 15.3 mmol) in dichloromethane (35 ml) at 0 °C. After stirring for 10 minutes at 0 °C, the reaction was allowed to warm to 25 °C and stirred for an additional 2 hours. The reaction was poured into water (50 ml) and extracted with two 30 ml aliquots of dichloromethane. The organic portions were combined and dried over magnesium sulfate. Evaporating the solvent left an oil, 3-methanesulfonyl methylfuran, used without further purification.

3-Methanesulfonyl methylfuran was added to a suspension of sodium theobromine (3.00 g, 14.8 mmol) in dimethylsulfoxide (35 ml) and the reaction stirred for 16 hours at 60 °C. The mixture was then poured into water (100 ml) and extracted with a 1:1 solution of ethyl acetate/dichloromethane (200 ml). The organic portion was dried over magnesium sulfate and the solvent evaporated, leaving a yellow solid. The yellow solid, purified by chromatography with silica using a methanol/dichloromethane eluant, produced 2.23 g of a white solid, compound no. 2504, (56% yield).

Example 3

A solution of dimethylsulfoxide (20 ml), sodium hydride (0.088 g, 3.5 mmol) and theobromine (0.63 g, 3.5 mmol) was stirred under an argon atmosphere. After 10 minutes, 2-bromoethyl)benzene (0.48 ml, 3.5 mmol) was added to this slurry. After 14 hours, water (25 ml) was added to the dimethylsulfoxide solution (with heat evolution) and this aqueous dimethylsulfoxide solution stirred for 20 minutes. The aqueous dimethylsulfoxide solution was extracted with three 50 ml aliquots of dichloromethane. The dichloromethane phases were combined and washed with three 50 ml aliquots of water. The organic phase was dried over sodium sulfate, filtered, and the solvent removed under vacuum, leaving a white residue. The white residue, recrystallized from dichloromethane/hexanes, yielded 0.57 g of a white solid, compound no. 2506 (57% yield).

Example 4

This example is a method of synthesis for inventive compound no. 2522. Over 30 minutes, a 2.0 M solution (30 ml) of butyllithium (60.0 mmol) in cyclohexane was added to a solution (300 ml) of furan (4.14 g, 60.9 mmol) in tetrahydrofuran at -15 °C and the reaction

stirred at -15 °C for 30 minutes and 0 °C for 90 minutes. After cooling to -78 °C, this solution was added via a canula over 50 minutes to a solution (462.3 ml) of 1,3-dibromopropane (12.3 ml, 120 mmol) in tetrahydrofuran (450 ml), also at -78 °C, and the reaction mixture stirred at -78°C for 1 hour and then allowed to warm to 25 °C over 5 hours. Saturated ammonium chloride (100 ml) and water (100 ml) were added to the reaction mixture and the mixture extracted with two 100 ml aliquots of diethyl ether. The combined organic extracts were dried over magnesium sulfate and the solvent evaporated, leaving a residue subsequently distilled at 8 mm Hg through a 25 cm vigreaux column to obtain 5.36 g of a colorless liquid, 2-(3-bromopropyl)-furan (47% yield, b.p. 70-73 °C).

2-(3-Bromopropyl)-furan (1.00 g, 5.30 mmol) was added to a suspension (15 ml) of sodium theobromine (1.10 g, 5.44 mmol) in dimethylsulfoxide and the reaction stirred for 16 hours at 60 °C. The mixture was then poured into water (50 ml) and extracted with three 30 ml aliquots of diethyl ether. The organic portions were combined, dried over magnesium sulfate and evaporated, leaving a slightly yellow solid. Recrystallizating the yellow solid from hot diethyl ether produce 1.31 g of a white solid, compound no. 2522 (86% yield).

Example 5

This example is a synthesis of inventive compound no. 2526 (see above for chemical name and structure). A 2.0 M solution (30 ml) of butyllithium (60.0 mmol) in cyclohexane was added to a solution (300 ml) of furan (4.14 g, 60.9 mmol) in tetrahydrofuran at -15 °C over 30 minutes and the reaction stirred at -15 °C for 30 minutes and at 0 °C for 90 minutes. After cooling this reaction mixture to -78 °C, this solution was added via a canula over 50 minutes to a solution (450 ml) of 1,6-dibromohexane (25.0 g, 102 mmol) in tetrahydrofuran, also at -78 °C, and the reaction stirred at -78 °C for 1 hour and then allowed to warm to 25 °C over 5 hours. Saturated ammonium chloride (100 ml) and water (100 ml) were added and the reaction mixture extracted with two 100 ml aliquots of diethyl ether. The combined organic extracts were dried over magnesium sulfate and the solvent evaporated, leaving a residue which was subsequently distilled at 0.5 mm Hg through a 25 cm vigreaux column to obtain 3.82 g of a colorless liquid, 2-(6-bromohexyl)-furan (30% yield, b.p. 85-87 °C).

2-(6-bromohexyl)-furan (1.00 g, 4.74 mmol) was added to a suspension (10 ml) of sodium theobromine (1.05 g, 5.21 mmol) in dimethylsulfoxide and the reaction stirred for 16 hours at 60 °C. The mixture was then poured into water (50 ml) and extracted with three 40 ml aliquots of ethyl acetate. The organic portions were combined and dried over magnesium sulfate and the solvent evaporated, leaving a slightly yellow solid. Recrystallization from hot ethyl acetate resulted in 1.20 g of a white solid, inventive compound no. 2526 (82% yield).

Example 6

This example is a method of synthesis for inventive compound no. 2533. A mixture (20 ml) of 1-chloro-3-cyclohexylpropane (0.8 ml, 5.0 mmol) and sodium theobromine (1.01 g, 5.0 mmol) in dimethylsulfoxide was stirred for 17 hours. The mixture was heated at 55 °C for 6 hours, and after cooling to ambient temperature, ether (50 ml) was added. The solution was washed with three 30 ml aliquots of water and dried over magnesium sulfate, and the ether was evaporated under vacuum. A crude residue was purified by flash chromatography over silica using an ethyl acetate eluant, producing 437 mg of compound no. 2533 (29% yield).

Example 7

This example is a method of synthesis for inventive compound no. 3527. 1,9-Dibromononane (8.58 g, 3.0 mmol) was added to a suspension (50 ml) of potassium phthalimide (5.55 g, 3 mmol) in dimethyl sulfoxide and stirred overnight. After 12 hours of stirring at room temperature, the reaction was poured into a separatory funnel containing 200 ml of water and extracted with three 100 ml aliquots of ethyl acetate. The organic extracts were combined, washed with water (100 ml) and brine (100 ml), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. A crude product obtained was further purified by flash chromatography over silica gel using a 30% ethyl acetate/hexane eluant, producing 8.21 g of N-(9-Bromononyl)phthalimide (77.8 % yield).

Sodium hydride (720 mg, 30 mmol) was added to a suspension (50 ml) of theobromine (4.5 g, 25 mmol) and N-(9-bromononyl)phthalimide (7.04 g, 20 mmol) in dimethyl sulfoxide. The resulting reaction mixture was stirred for 24 hours, after which, the reaction was poured into 300 ml of water and filtered. A crude product obtained was further purified by flash chromatography over silica gel using a 20% methanol/dichloromethane, to obtain 1.3 g of compound no. 3527 (14.4% yield).

Example 8

This example shows an inhibitive effect of inventive compounds nos. 1815, 1816, 2504, 2505, 2507, 2511, 2522, 2526 and 2531 on murine thymocyte proliferation stimulated by Concanavalin A (ConA) and interleukin-2 (IL-2). This assay is an *in vitro*, predictive model of a compounds' therapeutic potential in treating or preventing autoimmune, immune or inflammtory diseases. Procedurally, thymuses were obtained from normal, female Balb/C mice. The thymuses were dissociated and plated into 96-well plates at a density of 2×10^5 cells/well. ConA (0.25 mg/ml) and IL-2 (12.5 ng/ml) were added to the wells. Drug was added at various doses two hours prior to activation with ConA and IL-2. The cells were incubated for 4 days at 37 °C. On day 4, the cells were pulsed with tritiated thymidine and allowed to incubate for an

additional 4 hours. Harvested cells were analyzed for incorporated tritiated thymidine, determined using a liquid scintillation counter. Dose response curves were prepared from the assay results and used to calculate an IC50 value for each compound tested.

In representative dose response curves for compounds nos. 2504 and 2507, figure 1 illustrates the inhibitive effects of these compounds on proliferation of thymocytes stimulated with ConA and IL-2. Background counts, without addition of representative inventive compounds were about 170 cpm. Figure 1 illustrates an ability of the inventive compounds, particularly compound no. 2507, representative of ring substituted inventive compounds, to inhibit ConA/IL-2 stimulated proliferation at compound concentrations less than 100 μM. IC50 values experimentally calculated from dose response curves prepared for compounds tested are plotted in a bar graph of figure 2. These concentrations plotted are within known *in vivo* concentrations useful in treating disease.

Example 9

This example illustrates an ability of inventive compounds nos. 1815, 1816, 2504, 2504, 2507, 2511, 2522 and 2526 to inhibit proliferation of peripheral blood mononuclear cells (PBMC) in response to allogeneic stimulation. This *in vitro* mixed lymphocyte reaction (MLR) assay is useful in assessing biological activity of an inventive compound. Procedurally, PBMC were obtained by drawing whole blood from healthy volunteers in a heparinized container, the whole blood samples diluted with an equal volume of hanks balanced salt solution (HBSS).

This mixture was layered on a sucrose density gradient, such as a Ficoll-Hypaque® gradient (specific gravity 1.08), and centrifuged (1000 x g) for 25 minutes at no warmer than room temperature. PBMC were obtained from a band at a plasma-Ficoll interface, separated and washed at least twice in a saline solution, such as HBSS. Contaminating red cells are lysed, for example, by ACK lysis for 10 minutes at 37 °C, and the PBMC were washed twice in HBSS. The pellet of purified PBMC was resuspended in complete medium, such as RPMI 1640 plus 20% human inactivated serum.

Proliferative response of PBMC to allogeneic stimulation was determined in a two-way MLR performed in a 96-well microtiter plate. Approximately 10^5 test-purified PBMC in 200 μl complete medium were co-cultured with approximately 10^5 autologous (control culture) or allogeneic (stimulated culture) PBMC. Allogeneic cells were from HLA disparate individuals. Varying doses of compounds nos. 1815, 1816, 2504, 2504, 2507, 2511, 2522 and 2526 were added simultaneously upon addition of cells to the microtiter plate. The cultures were incubated for 6 days at 37 °C in a 5% CO_2 atmosphere, after which time, tritiated thymidine was added (for example, 1 μCi/well of 40 to 60 Ci/mmole) and proliferative

inhibition was assessed by determining amount of tritiated thymidine taken up, using liquid scintillation counting.

Figures 3, 4 and 5 are plotted graphs of compound concentrations (μM) and inhibition (as a function of incorporated thymidine, cpm) for compounds nos. 2504, 2522 and 2526, respectively. Figure 5 illustrates a most pronounced inhibition of PBMC proliferation. At concentrations less than 100 μM, compound no. 2526 significantly inhibited incorporation of thymidine. Similarly, although to lesser degrees in comparison to compound no. 2526, figures 4 and 5 illustrate inhibitive characteristics of inventive compounds nos. 2504 and 2522, in this MLR assay at compound concentrations less than 250 μM and 100 μM, respectively. Figure 6 is a bar graph of experimentally calculated IC50 values for compounds tested in this MLR assay. Some IC50 values are in the 250 μM range, while others, such as 2507, exhibit substantial potency with an IC50 value of about 20 μM. These results suggest specificity of the inventive compounds for particular disease treatments.

In a parallel assay, conducted simultaneously with the MLR assay above, the number of viable cells were assessed after a six day incubation with the inventive compound to determine concentrations at which the compounds might be cytotoxic. This viability assay procedure was identical to the above procedure with the exception that predetermined concentrations of compound were used and instead of using tritiated thymidine, the cells in the 96 well plate were well mixed with an aliquot of added trypan blue solution. Viable cells will exclude this dye however dead cells take up the dye and appear blue under a microscope. Both live and dead cells were counted and compared to a control untreated with inventive compound.

This control normally had a viability ranging from 75 to 80%. In general, viability was assessed at 100μM. Viability results, shown in a bar graph of figure 7, illustrate that the inventive compounds were generally not cytotoxic at 100 μM. The most potent inventive compound represented, no. 2507, was not cytotoxic at 100 μM but this concentration is well above its IC50 value indicating the presence of a significant therapeutic window.

Example 10

This example illustrates inhibitive effects of inventive compounds nos. 2507, 2511, 2522, 2526, and 2531 (see above for chemical names and structures) on proliferation of human stromal cells stimulated with platelet derive growth factor (PDGF). This assay is a model for restinosis and useful in predicting a compound's potential as a treatment for atherosclerosis and coronary artery disease.

Procedurally, stromal cells were starved in serum-free media for one day and then stimulated with 50 ng/ml PDGF-BB. Inventive compounds were were added at predetermined concentrations one hour prior to PDGF stimulation. Tritiated thymidine was added for one day

at the time of PDGF stimulation and the cells were harvested and counted by liquid scintillation counting 24 hours later. Background counts (*i.e.*, starved cells) were approximately 1% of control levels. Figure 8, reporting results from this assay, shows that all inventive compounds tested were active in this predictive *in vitro* model. Inventive compounds nos. 2507 and 2526 illustrate more potent activity, in comparison to other representative compounds tested.

Example 11

This example illustrates inhibitive effects of the inventive compounds on Balb/3T3 cell proliferation in response to platelet derived growth factor (PDGF) stimulation.

Disregulated PDGF-proliferative response has been linked to a variety of diseases, including, *e.g.*, restenosis, atherosclerosis, fibrosis, and tumor cell angiogenesis. Balb/3T3 cells respond vigorously to PDGF stimulation, and are useful *in vitro* models for further study of PDGF-induced proliferation. In an assay useful in determining whether a compound would be useful in treating diseases characterized by this or similar disregulated proliferative responses, research indicates that the inventive compounds inhibit PDGF-induced proliferation of Balb/3T3 cells.

Balb/3T3 cells were plated in low serum-containing medium for 24 hours prior to stimulation with various concentrations of inventive compound no. 3527. PDGF was added at varying concentrations along with tritiated thymidine. The cells were allowed to incubate for one day, following addition of PDGF and thymidine. 24 hours later, the cells were harvested and counted by liquid scintillation counting. Figure 9 reports data obtained in this proliferation assay. The results illustrate that compound no. 3527 inhibits PDGF-stimulated proliferation of Balb/3T3 cells at concentrations less than 30 μM, indicating that the inventive compounds are candidates for treating or preventing restenosis, atherosclerosis, fibrosis, tumor cell angiogenesis and other similar diseases.

In conjunction with the Balb/3t3 proliferation assay, a related viability assay was conducted to assess the cytotoxicity of compounds which inhibit proliferation in this system. The assay protocol was identical to that performed above except that tritiated thymidine was not added after the 24 hour incubation with PDGF. Subsequent to incubation, a 10 μM solution of BCECF was added and the cells incubated for 30 minutes at 37 °C. Following this incubation, BCECF was replaced with PBS and the plate read for flourescence in a Millipore "cytofluor". Data obtained was plotted as a percent of control versus concentration of inventive compound tested. Figure 10 represents the results of this viability assay. The compound tested, representative of compounds of the invention, was not cytotoxic to any cells (as compared with a control value of 100 %) at concentrations that compound no. 3527 inhibited proliferation.

Example 12

This example illustrates inhibitive effects of inventive compounds nos. 2507, 2511, 2522 and 2526 (see above for chemical name and structure) on inhibition of blast formation from human lymphocytes stimulated by IL-2 or an anti-CD3 antibody. Results from this assay are shown in figure 11. This is a human *in vitro* assay providing an additional basis for assessing whether tested compounds have immunosuppressive activity. As shown in figure 11, all inventive compounds tested demonstrate some immunosuppressive activity of blastogenesis stimulated by either IL-2 or anti-CD3 with IC50 values at or below 50 μM.

Example 13

This example illustrates an ability of inventive compound no. 2507 to inhibit adherence of specific cells to human umbilical vein endothelial cells (HUVEC) stimulated with IL-1β. Cell-surface receptor signaling has an important role in aggravating or promoting particular immune, inflammatory, cancers and other diseases. This adhesion assay is useful in showing an ability of an inventive compound to inhibit adhesion of a specific cell to other cells in this signaling phenomenon, and thus in predicting therapeutic potential of the inventive compounds.

Two days prior to conducting the assay procedure, HUVEC were plated at 4000 cells/well. After two days, HUVEC were stimulated overnight with IL-1β (20 ng/ml). THP-1 (a human acute monocytic leukemia cell line) cells were prestained with 2,7-bis-(2-carboxyethyl)-5(and-6)carboxyflourescein,acetoxymethyl ester (BCECF--a compound that when cleaved by esterases, yields a flourescent product, thus providing a measure of cell number) and added at 250,000 per well in RPMI containing 1% fetal calf serum. Proper controls were maintained and simultaneously analyzed, both in the absence of inventive compound and IL-1β. The cells were allowed to adhere for 20 minutes at 37 °C, after which time, the plate was inverted and spun at 800 rpm. The wells were washed once with PBS and resuspended in 100 μl PBS prior to reading fluorescence on a Millipore fluorescence plate reader. Data was recorded as percent adherence of THP-1 cells to HUVEC at selected concentrations of compound no. 2507.

Figures 12 reports plotted results of data obtained in this assay. Figure 12 shows that compound no. 2507 inhibited THP-1 adhesion to IL-1β stimulated HUVEC. In all assay conditions, the representative, inventive compounds inhibit adherence at compound concentrations less than 10 μM, suggesting that the compounds tested, as representatives of all inventive compounds, possess therapeutic potential in diseases exhibiting biologic characteristics these assays are designed to model.

Example 14

This example illustrates the effect of CT2507 in an *ex vivo* human TNF model, a model predictive of compounds useful in treatment and prevention of septic shock and sepsis syndrome. In this assay, LPS was added to whole blood obtained from normal volunteers to trigger a dose-dependent synthesis and extracellular release of TNF according to Desch et al. (*Lymphokine Res.* 8:141, 1989). The *ex vivo* model examines whether a compound will block LPS-mediated release of TNF from monocytes in whole blood. Results in this assay are illustrated in figure 13. As shown at the concentrations tested, inventive compound no. 2507, representative of ring-substituted inventive compounds, blocked TNF release in a dose-dependent fashion.

Example 15

In a procedure similar to that followed in example 14, this example illustrates that inventive compounds nos. 1424, 2504 and 2507 inhibit lipo-protein saccharide (LPS)-induced TNF release in whole human blood, suggesting that the inventive compounds would be useful in treating and/or preventing septic shock and sepsis syndrome.

Describing a protocol in more detail, whole blood was collected from a healthy human donor into Vacutainer tubes containing ACD citrate as anti-coagulant. The compounds tested were diluted in RPMI medium and 5µl of the dilute concentrations placed in tubes containing 225µl of whole blood. The tubes were mixed and incubated for no more than 1hour at 37 °C. LPS Salmonella abortus equi (commercially available from Sigma) is diluted in RPMI and the dilute samples added to the whole blood/compound samples at 20 µl per tube (10ng/ml final concentration). The tubes are again mixed and incubated for an additional 4-6 hours at 37 °C. Activity is stopped by adding 750µl of RPMI to each tube, centrifuging and removing the cells. Supernatants are collected and stored overnight at 4 °C. The supernatant samples are assayed for TNF release using immunoassay kits (available commercially from Biosource International, Camarillo, CA).

Data collected and reported was plotted as percent inhibition (of a control at 100%) versus concentration of compound tested. A compound showing 0% is not inhibiting LPS-induced TNF release, while a compound showing 40% is inhibiting 40% of the maximum, control LPS response. Figure 14 reports these results for compounds nos. 1424, 2504 and 2507. Each compound tested inhibited TNF release up to between about 55 and 65% at concentrations less than 25 µM. These data indicate that the inventive compounds are potential therapeutic agents for treating or preventing diseases mediated by inhibiting or preventing TNF secretion.

What is claimed is:

1. A therapeutic compound, including resolved enantiomers, diastereomers, hydrates, salts, solvates and mixtures thereof, having the formula:

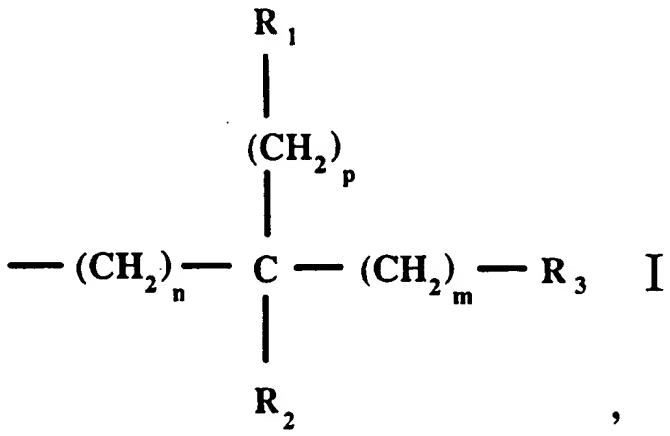
$$\text{CORE MOIETY} - (R)_j$$

wherein:

j is an integer from one to three;

the core moiety is non-cyclic or cyclic; and

R is selected from the group consisting of hydrogen, halogen, hydroxyl, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups and formula I:



wherein:

n is an integer from one to twenty;

m and p are independently zero or an integer from one to twenty;

R_1 is selected from the group consisting of hydrogen, halogen, hydroxide, and substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy, $C_{(2-10)}$ alkenyl, and a ring group having at least one four- to seven-membered ring;

R_2 is selected from the group consisting of hydrogen, halogen, hydroxide, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy and $C_{(2-10)}$ alkenyl;

R_3 is selected from the group consisting of hydrogen or a substituted or unsubstituted ring group having at least one four- to seven-membered ring;

at least one of R_1 or R_3 is the ring group; and

a sum of either (n + m) or (n + p), corresponding to a respective R_1 or R_3 ring group is not greater than nineteen, with the proviso that when the core moiety is xanthine or a substituted derivative thereof, n is not less than two.

2. The compound according to claim 1, wherein at least one of $(CH_2)_n$, $(CH_2)_m$ or $(CH_2)_p$ is substituted by a hydrogen, halogen, hydroxide, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic group; has one or two unsaturated bonds; or is interrupted by at least one oxygen atom.

3. The compound according to claim 2, wherein the one or two unsaturated bonds are in a *cis* configuration.

4. The compound according to claim 1, wherein n is an integer from about three to about eighteen.

5. The compound according to claim 1, wherein n is an integer from about three to about seven.

6. The compound according to claim 1, wherein m is zero and R_3 is the ring group.

7. The compound according to claim 1, wherein when: I) R, R_1 or R_2 are one of substituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl; II) R_1 or R_2 are substituted $C_{(1-10)}$ alkoxy; III) R is a substituted cyclic or heterocyclic group; or IV) R_3 or R_4 is a substituted ring group having at least one four- to seven-membered ring, R, R_1, R_2 or R_4 substituenta are selected from the group consisting of amide, primary, secondary and tertiary amine, $C_{(2-8)}$ alkenyl, $C_{(1-8)}$ alkyl, $C_{(1-8)}$ alkoxyalkyl, azide, carbonate, carbonyl, carboxylic acid, cyanide, $C_{(1-8)}$ haloalkyl, isocyanate, isothiocyanate, phosphate, phosphonate, primary, secondary or tertiary alcohol, sulfonate, sulfone, sulfoxide, thioamide, thiocarbonate, thioester, thiolester, thiol, thiourea and urea.

8. The compound according to claim 7, wherein the $C_{(1-8)}$ haloalkyl substituent is a mono-, di- or tri-substituted haloalkyl.

9. The compound according to claim 7, wherein the alcohol is selected from the group consisting of diol, methanol, butanol, 1-cyclopentanol, ethanol, 2-ethyl-3-methyl-1-propanol, pentanol, propanol and methylcyclohexanol.

10. The compound according to claim 1, wherein the R cyclic or heterocyclic groups, or R_1 or R_3 ring group is selected from the group consisting of: anthracene, bicyclo[4.4.0]decane, bicyclo[2.2.1]heptane, bicyclo[3.2.0]heptane, bicyclo[4.1.0]heptane, bicylo[2.2.1]hexane, bicyclo[4.3.0]nonane, bicyclo[2.2.2]octane, biphenyl, cyclopentadiene, cyclopentane, cyclobutane, cyclobutene, cycloheptane, cyclohexane, cyclooctane and cyclopropane, 1,2-diphenylethane, fluorene, indene, phenyl, quinone, terphenyl, napthalene, phenanthrene, terphenyl, toluene, xylene and derivatives thereof.

11. The compound according to claim 1, wherein the R cyclic or heterocyclic groups, or R_1 or R_3 ring group is selected from the group consisting of azetidine, benzofuran, benzothiophene, carbazole, furan, glutarimide, indole, isoquinoline, lactam, lactone, oxazole, oxetane, oxirane, pyrrolidine, pyran, piperidine, pyridine, pyrrole, quinoline, tetrahydrofuran, tetrahydropyran, tetrahydrothiophene, thiophene and derivatives thereof.

12. The compound according to claim 1, wherein the non-cyclic core moiety is selected from the group consisting of acetamide, amide, amine, one or two amino acids, carboxide, ester, terminal halogen or hydrogen atom, hydroxide, glutaric acid, glycine derivative, ketone, phosphate, phosphonate, sulfate, sulfonate, sulfone, sulfoxide, simple ionic functional group, thiol and thiolester.

13. The compound according to claim 1, wherein the cyclic core is at least one five- to seven-member, non-heterocyclic ring or heterocycle

14. The compound according to claim 1, wherein the cyclic core has from one to three, five- to six-membered ring structures in a predominantly planar configuration.

15. The compound according to claim 13, wherein the non-heterocyclic ring core is selected from the group consisting of substituted or unsubstituted benzene; biphenyl; cyclohexane; cyclohexanedione; cyclopentanedione; napthlalene; phenol; quinone; salicylic acid and derivatives thereof; stilbene and tricyclododecane.

16. The compound according to claim 13, wherein the heterocyclic core is selected from the group consisting of substituted or unsubstituted barbituric acid; benzamide; lactam; glutarimide; homophthalimide; hydrophthalimide; imidazole; imidazole amide; indomethacin; isocarbostyril; lumazine; N-alkylheterocyclic; N-heterocyclic; pteridine; pthalimide; piperidine; pyridine; pyrimidine; pyrrole amide; quaternized N-heterocyclic; quinolizinedione; quinazolinone; quinoline; recorsinol; succinimide; theobromine; thymine; triazine; uric acid; uracil; vitamins A, E or K and xanthine.

17. The compound according to claim 13, wherein the non-heterocyclic ring is selected from the group consisting of substituted or unsubstituted 1,3-cyclohexanedione, 1,3-cyclopentanedione; 1,3-dihydroxynaphthalene and orthophenol.

18. The compound according to claim 13, wherein the heterocyclic core is selected from the group consisting of $C_{(1-6)}$ alkyl-substituted thymine; $C_{(1-6)}$ alkyl-substituted uracil; 1,3-dihydroxynapthalene; 3,3-dimethylglutarimide; dihydrothymine; 2,4-dioxohexahydro-1.3.5-tetrazine; hexahydrophthalimide; homophthalimide; 2-hydroxypyridine; β-ionone as vitamin A methylbarbituric acid; 2,6,6-methyl-1-cyclohexene-1-acetaldehyde as vitamin A; methyldihydroxypyrazolopyrimidine, specifically 1,3-dimethyldihydroxypyrazolo[4,3-d]pyrimidine;1-methyl-5,6-dihydrouracil; 1,7-dimethylxanthine, 3,7-dimethylxanthine; 7-methylhypoxanthine; 1-methyllumazine; 3-methyl-7-methylpivaloylxanthine;

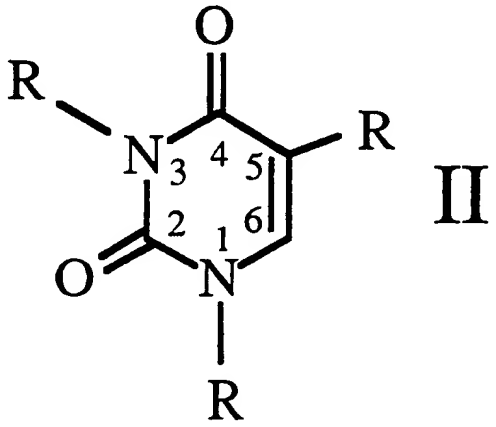
methylpyrrolopyrimidine; 1-methylpyrrolo [2,3-d] pyrimidine; 1-methyl-2,4(1H,3H)-quinolizinedione (1-methylbenzoyleneurea); methylthymine; 1-methyluracil; 3-methylxanthine; orotic acid; prostacyclin; 1-pyrrole amides; 2-pyrrole amides; 3-pyrrole amides; quinazolin-4(3H)-one; 1,2,3,4-tetrahydroisoquinolone; tetrahydrophthalimide; sulindac; uracil fused to naphthalene; 5- and/or 6-position substituted uracils; tetralone to vitamin K; and 8-substituted xanthines, having substituents such as N or S.

19. The compound of claim 13, wherein the heterocyclic core is selected from the group consisting of substituted or unsubstituted 3,7-dimethylxanthine, glutarimide, 3-methyl-7-pivoloylxanthine, methylthymine, methyluracil, 3-methylxanthine, tetrahydrophthalimide, thymine, uracil and xanthine.

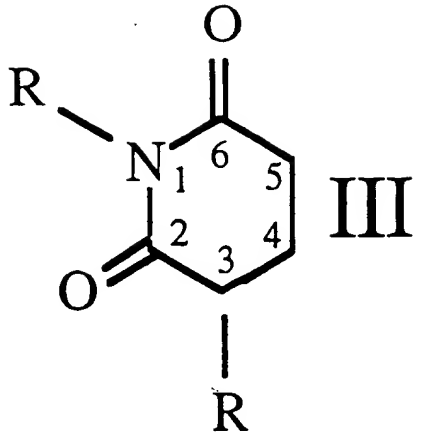
20. The compound according to claim 19, wherein R having formula I is bonded to an: 1) N_1 nitrogen of glutarimide; 2) N_1 nitrogen xanthine, wherein N_3 and N_7 xanthine nitrogens are independently substituted by a member selected from the group consisting of hydrogen, $C_{(1-6)}$ alkyl, fluoro, chloro and amino; 3) N_3 nitrogen of a or methylthymine; or 4) N_3 nitrogen of uracil.

21. The compound according to claim 19, wherein R having formula I are bonded to N_1 and N_3 xanthine nitrogens and the N_7 xanthine nitrogen is substituted by a member selected from the group consisting of hydrogen, methyl, fluoro, chloro and amino.

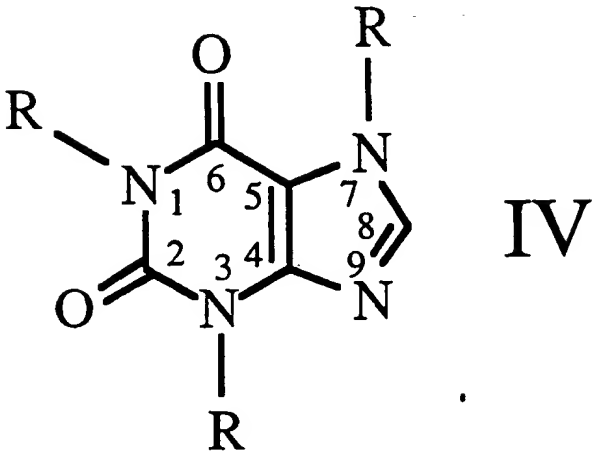
22. The compound according to claim 1, wherein the compound is selected from the group consisting of formulas II, III and IV:



II

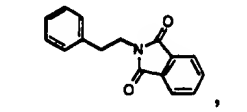


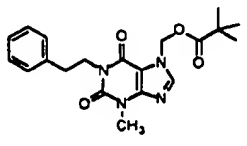
III

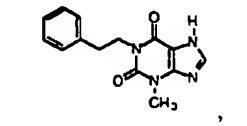


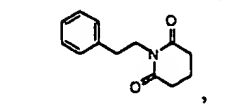
IV

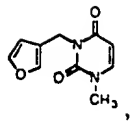
23. The compound according to claim 1, wherein the compound is selected from:

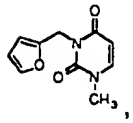


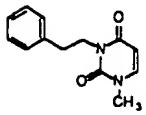


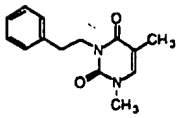


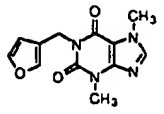


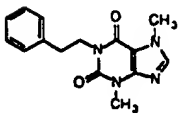
 ,

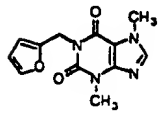
 ,

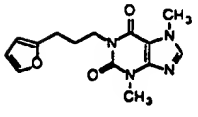
 ,

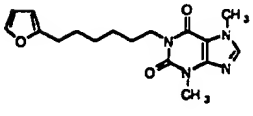
 ,

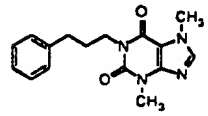
 ,

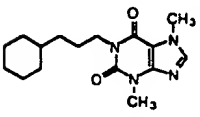
 ,

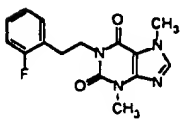
 ,

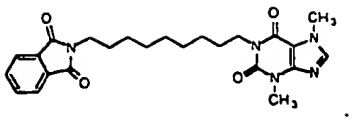
 ,

 ,

 ,

 ,

 , and



24. A pharmaceutical composition comprising a compound according to claim 1 and a suitable carrier, diluent or excipient.

25. The pharmaceutical composition of claim 24, wherein the composition is formulated for parenteral, topical or oral administration or for inhalation.

26. A method of synthesizing a compound having a formula:

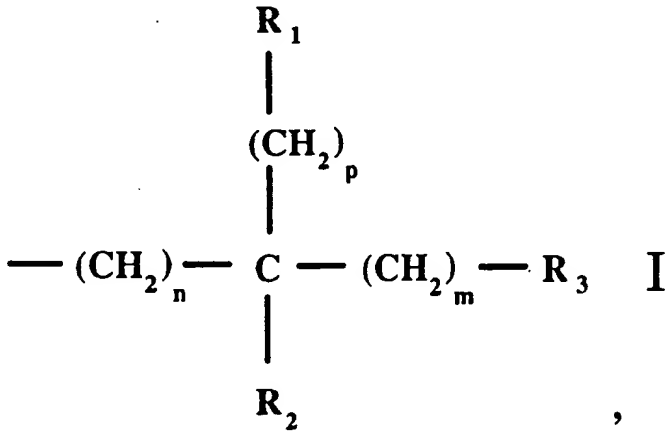
$$\text{CORE MOIETY} - (R)_j$$

wherein:

j is an integer from one to three;

the core moiety is non-cyclic or cyclic; and

R is selected from the group consisting of hydrogen, halogen, hydroxyl, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups and formula I:



wherein:

n is an integer from one to twenty;

m and p are independently zero or an integer from one to twenty;

R_1 is selected from the group consisting of hydrogen, halogen, hydroxide, and substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy, $C_{(2-10)}$ alkenyl, and a ring group having at least one four- to seven-membered ring;

R_2 is selected from the group consisting of hydrogen, halogen, hydroxide, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy and $C_{(2-10)}$ alkenyl;

R_3 is selected from the group consisting of hydrogen or a substituted or unsubstituted ring group having at least one four- to seven-membered ring;

at least one of R_1 or R_3 is the ring group; and

a sum of either (n + m) or (n + p), corresponding to a respective R_1 or R_3 ring group is not greater than nineteen, with the proviso that when the core moiety is xanthine or a substituted derivative thereof, n is not less than two, comprising:

preparing a cyclic intermediate from a compound comprising a cyclic functional group and a carbon chain of predetermined length to comprise a side arm of the inventive compound; and

reacting the cyclic intermediate and a core-containing compound.

27. The method of claim 26, wherein preparing a cyclic intermediate comprises obtaining an anion of the compound comprising a cyclic functional group and reactiong the anion with the carbon chain to obtain the cyclic intermediate.

28. The method of claim 26, wherein reacting the cyclic intermediate and a core-containing compound comprises obtaining an anion of the core-containing compound and allowing the anion to react with the cyclic intermediate.

29. The method of claim 28, wherein obtaining an anion comprises mixing the core-containing compound with a suitable base, solvent and cyclic intermediate.

30. A method for treating or preventing acute and chronic inflammatory diseases, AIDS and AIDS related complex, alcoholic hepatitis, allergies due to degranulation of mast cells and basophils, angiogenesis, asthma, atherosclerosis, autoimmune thyroiditis, coronary artery disease, glomerula nephritis, hair loss or baldness, HIV-associated dementia, inflammatory bowel disease, insulin dependent diabetes mellitus, lupus, malignancies, multiple sclerosis, myelogenous leukemia, organ or hematopoietic in response to cytotoxic therapy, osteoarthritis, osteoporosis, peridontal disease, premature labor secondary to uterine infection, psoriasis, restenosis, rheumatoid arthritis, sleep disorders, septic shock, sepsis syndrome, scleroderma, stroke and transplant rejection in a mammal in need of such treatment, comprising administering an effective amount of a compound having the following formula:

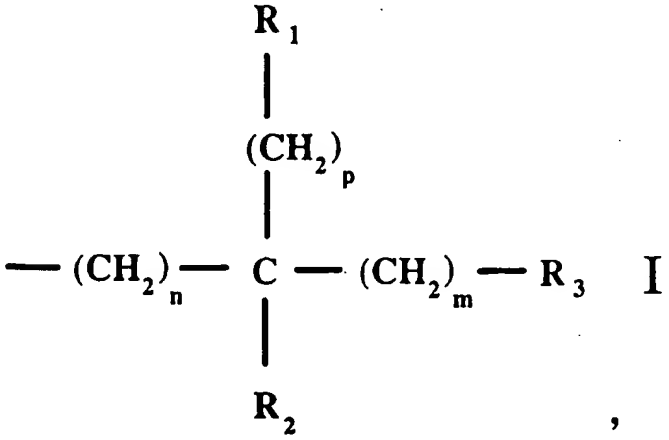
$$\text{CORE MOIETY} — (R)_j$$

wherein:

j is an integer from one to three;

the core moiety is non-cyclic or cyclic; and

R is selected from the group consisting of hydrogen, halogen, hydroxyl, amino, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(2-10)}$ alkenyl, cyclic or heterocyclic groups and formula I:



I

wherein:

n is an integer from one to twenty;

m and p are independently zero or an integer from one to twenty;

R_1 is selected from the group consisting of hydrogen, halogen, hydroxide, and substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy, $C_{(2-10)}$ alkenyl, and a ring group having at least one four- to seven-membered ring;

R_2 is selected from the group consisting of hydrogen, halogen, hydroxide, substituted or unsubstituted $C_{(1-10)}$ alkyl, $C_{(1-10)}$ alkoxy and $C_{(2-10)}$ alkenyl;

R_3 is selected from the group consisting of hydrogen or a substituted or unsubstituted ring group having at least one four- to seven-membered ring;

at least one of R_1 or R_3 is the ring group; and

a sum of either (n + m) or (n + p), corresponding to a respective R_1 or R_3 ring group is not greater than nineteen.

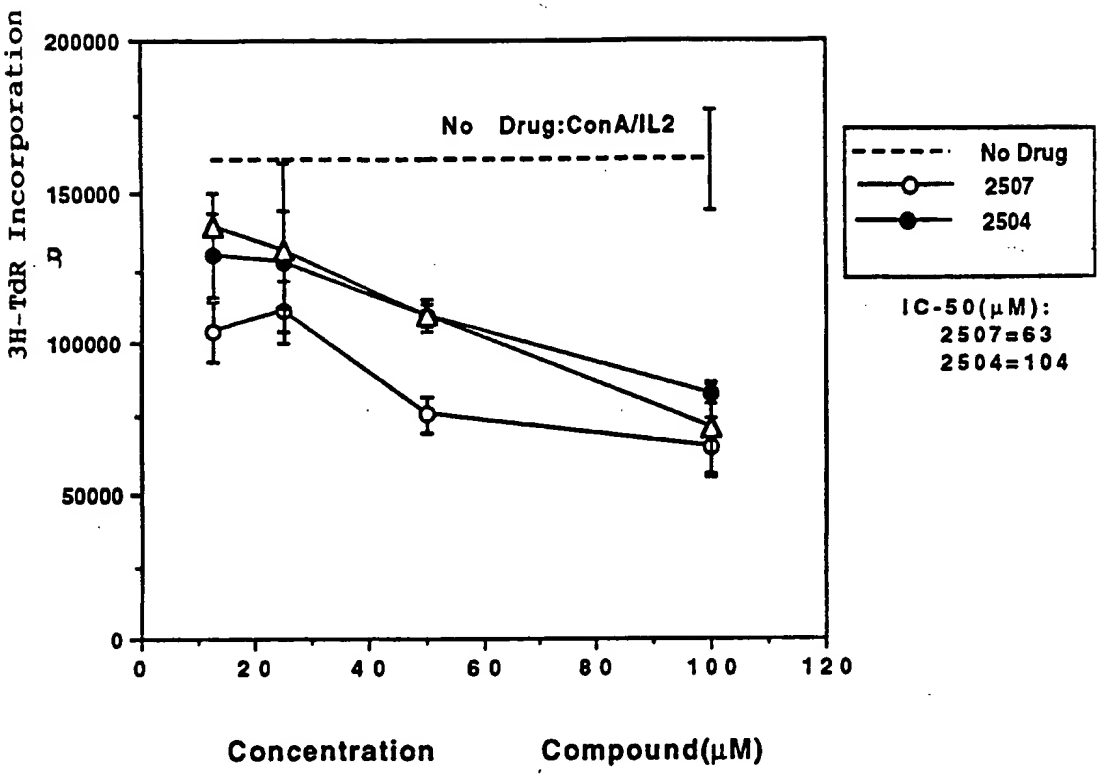

3H-TdR Incorporation
200000
150000
100000
50000
0
No Drug:ConA/IL2
0
20
40
60
80
100
120
Concentration Compound(μM)
No Drug
2507
2504
IC-50(μM):
2507=63
2504=104

FIGURE 1

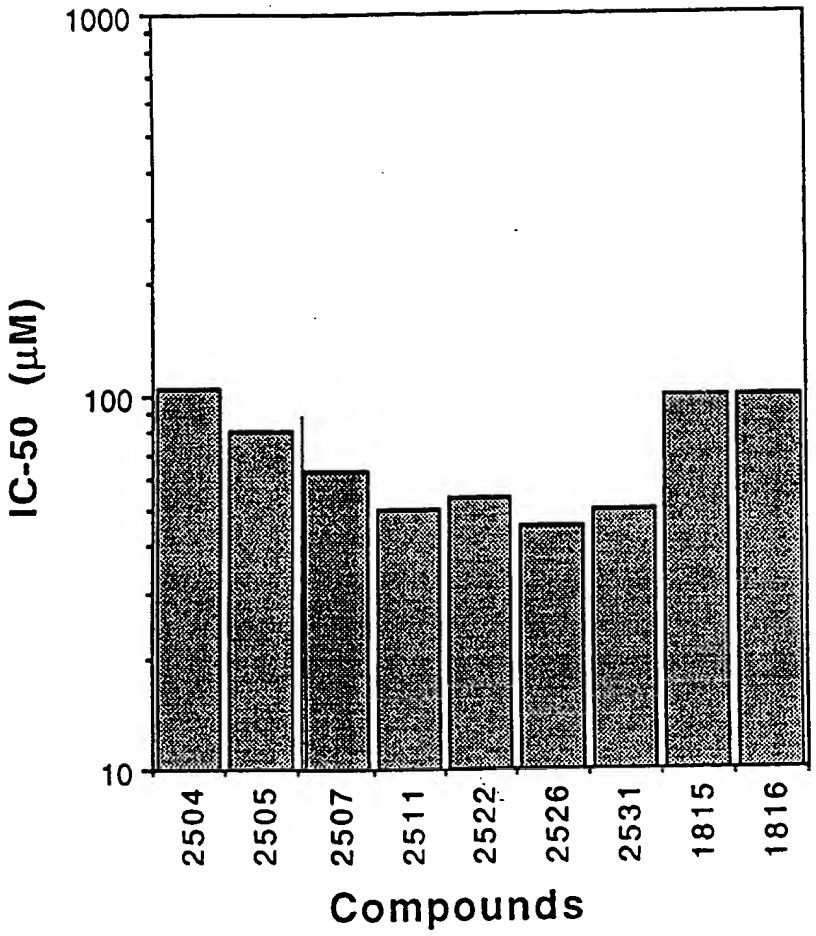

1000
100
10
IC-50 (μM)
2504
2505
2507
2511
2522
2526
2531
1815
1816
Compounds

FIGURE 2

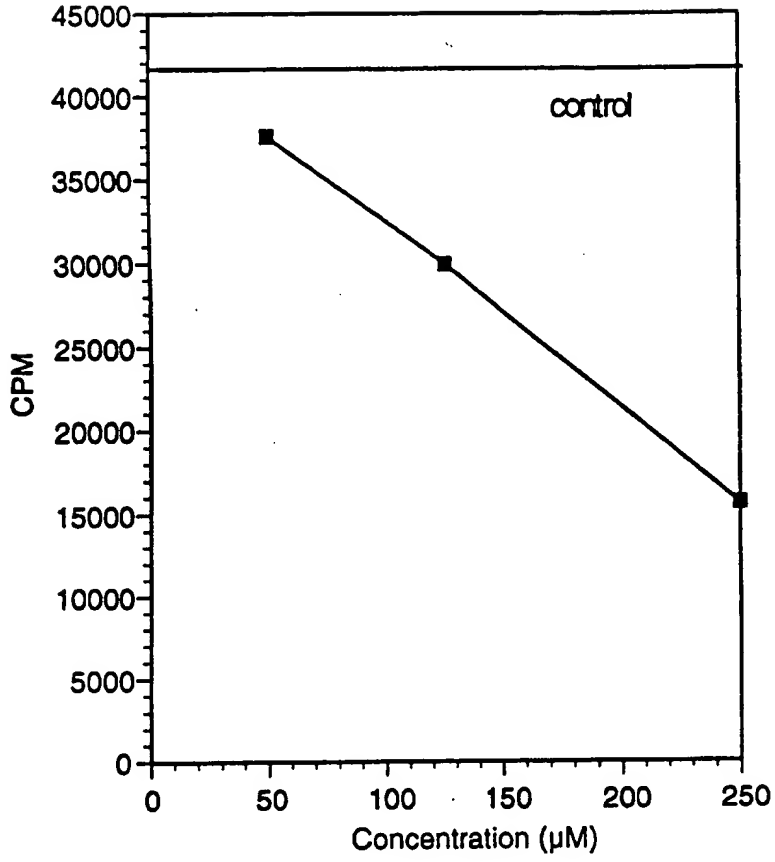
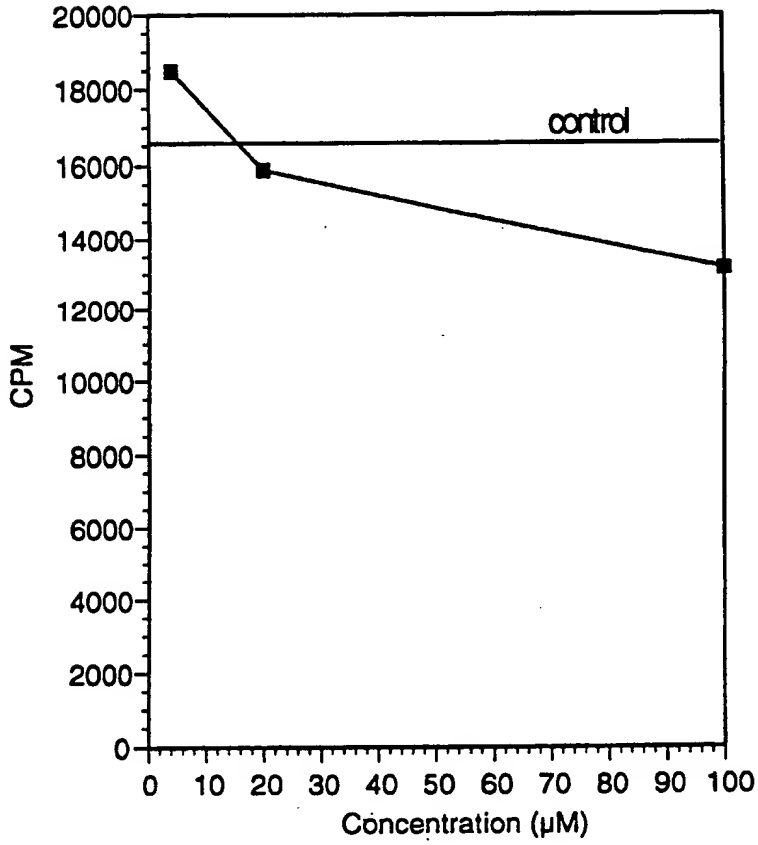
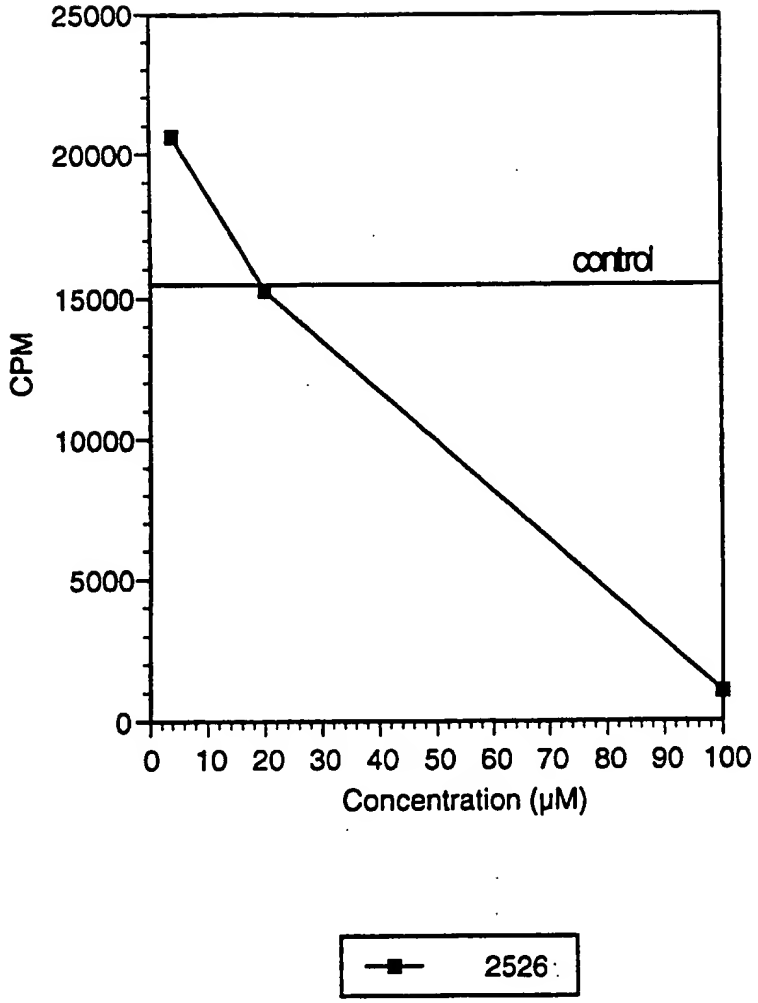

45000
40000
35000
30000
25000
20000
15000
10000
5000
0
CPM
control
0
50
100
150
200
250
Concentration (μM)
2504
IC-50 200μM

FIGURE 3



IC-50 > 100μM

FIGURE 4



IC-50 60μM

FIGURE 5

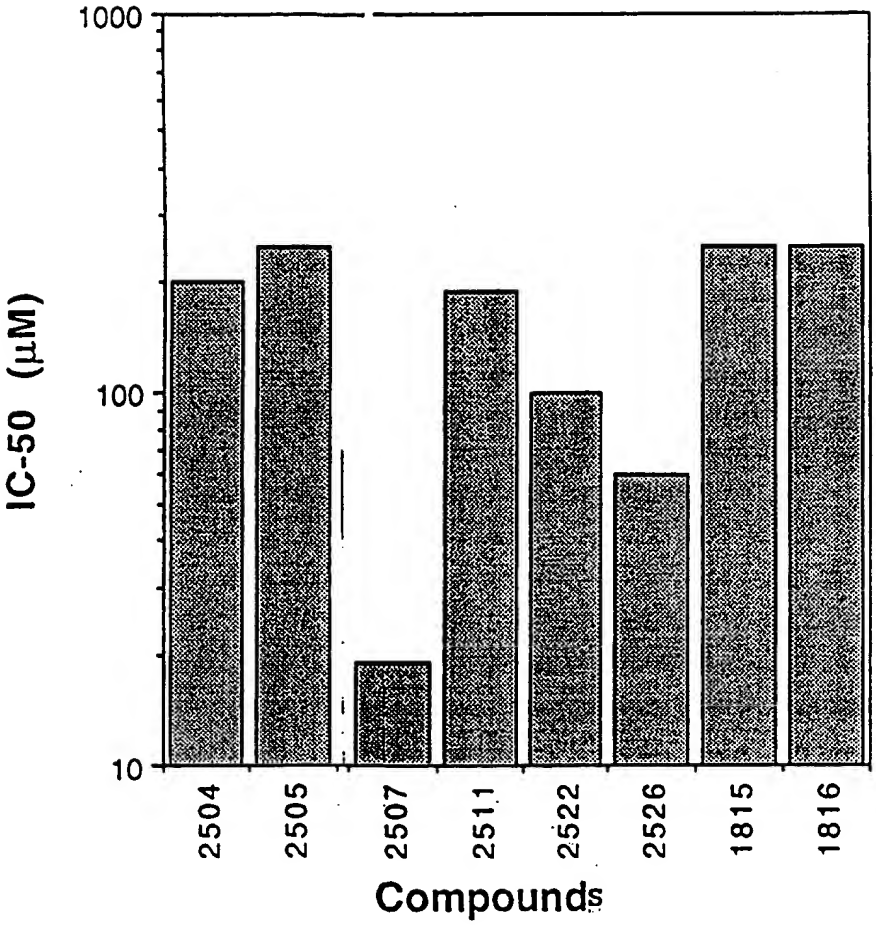

1000
100
10
IC-50 (μM)
2504
2505
2507
2511
2522
2526
1815
1816
Compounds

FIGURE 6

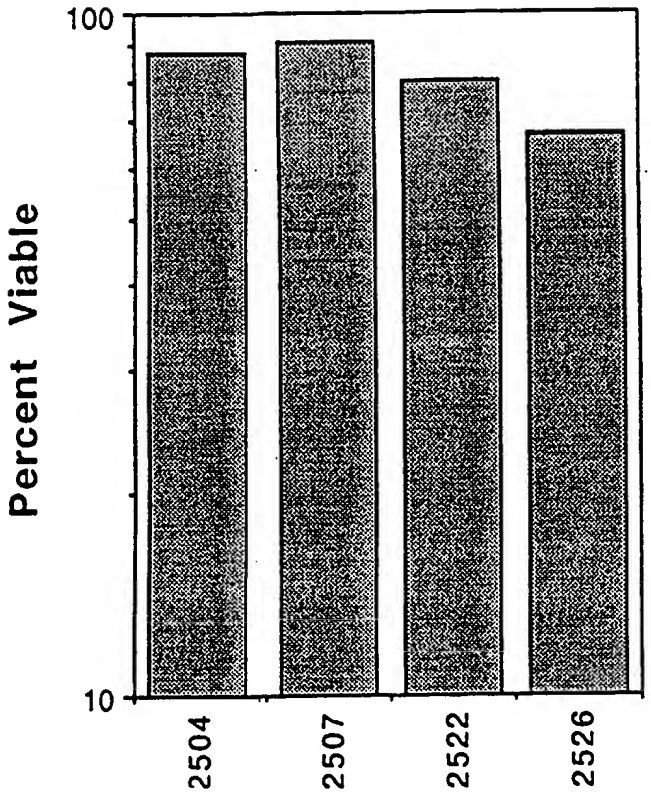


FIGURE 7

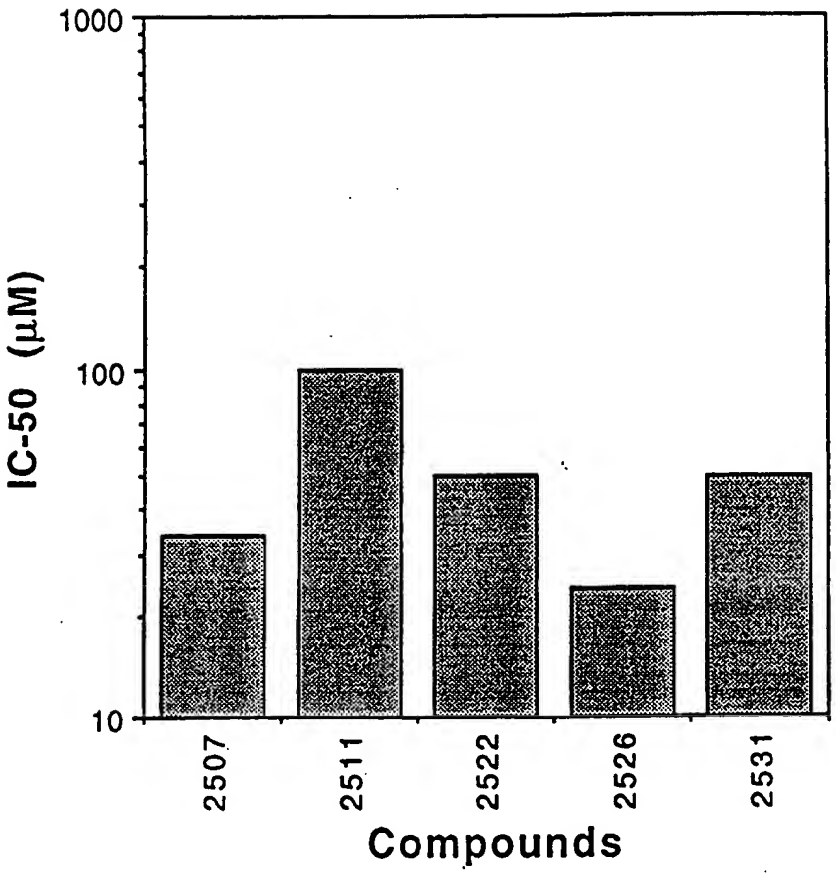


FIGURE 8

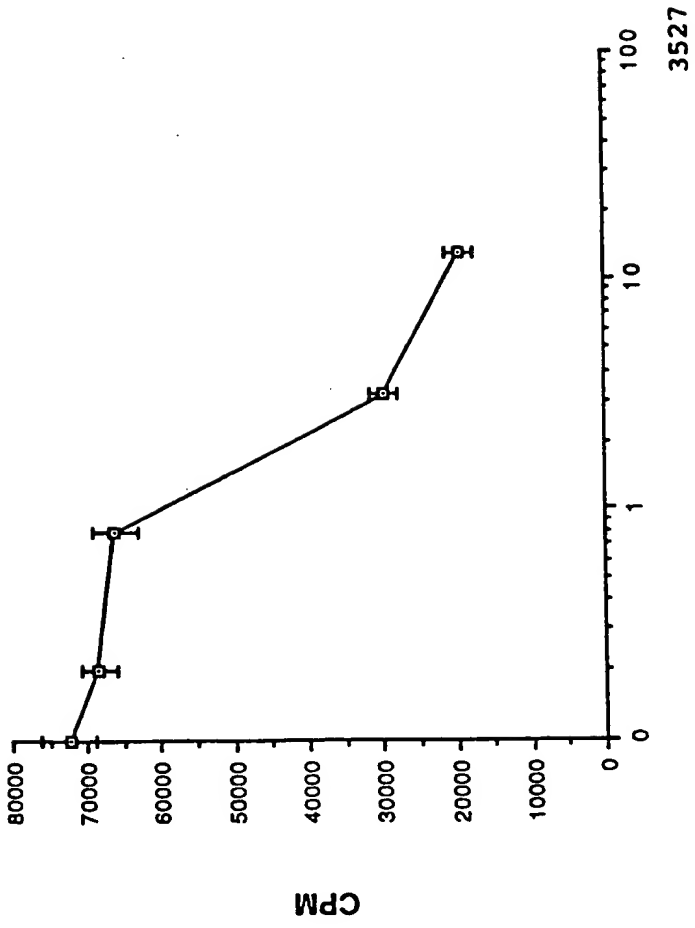

3527
CONC[μM]
0
1
10
100
CPM
0
10000
20000
30000
40000
50000
60000
70000
80000

FIGURE 9

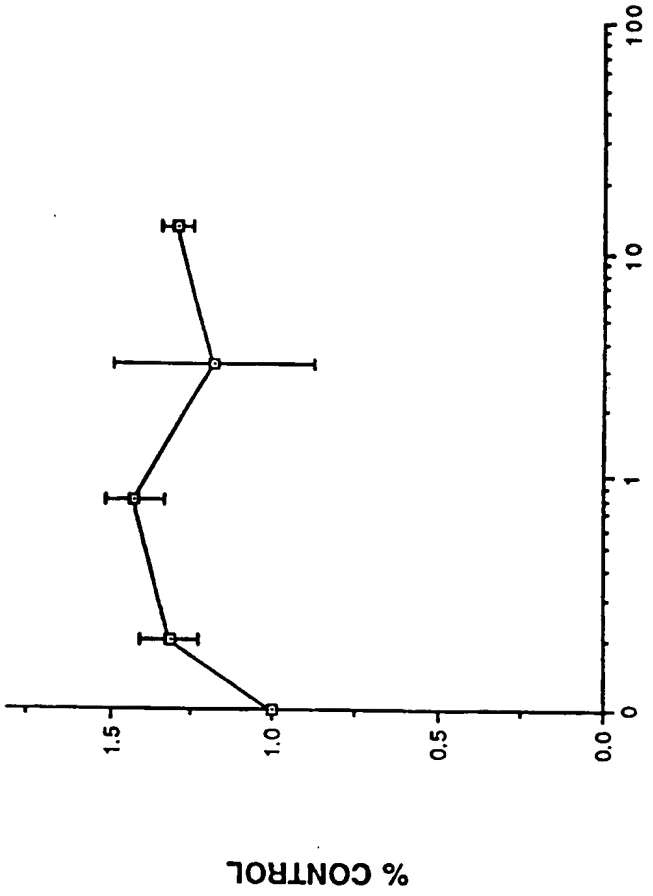


FIGURE 10

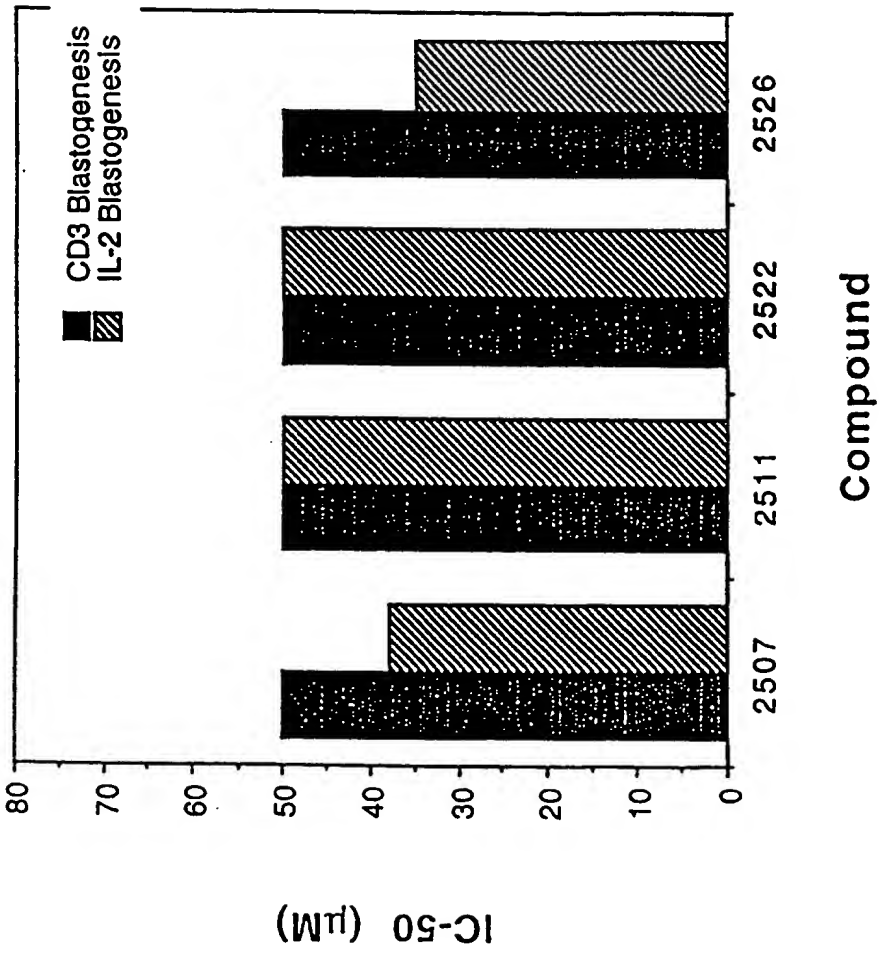

CD3 Blastogenesis
IL-2 Blastogenesis
IC-50 (μM)
0
10
20
30
40
50
60
70
80
2507
2511
2522
2526
Compound

FIGURE 11

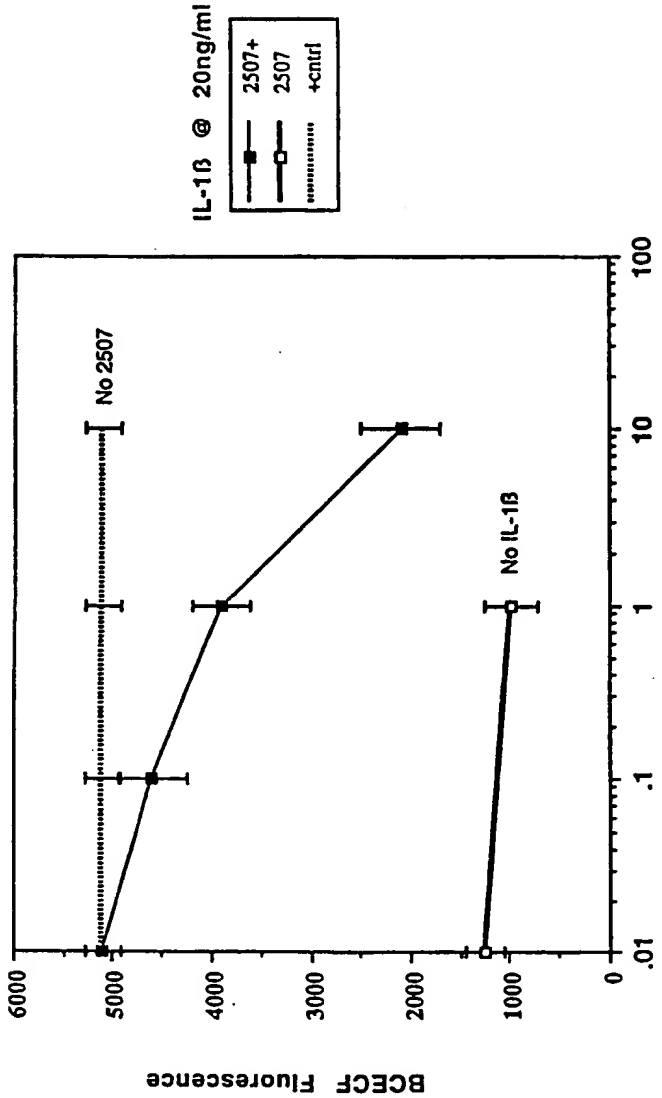
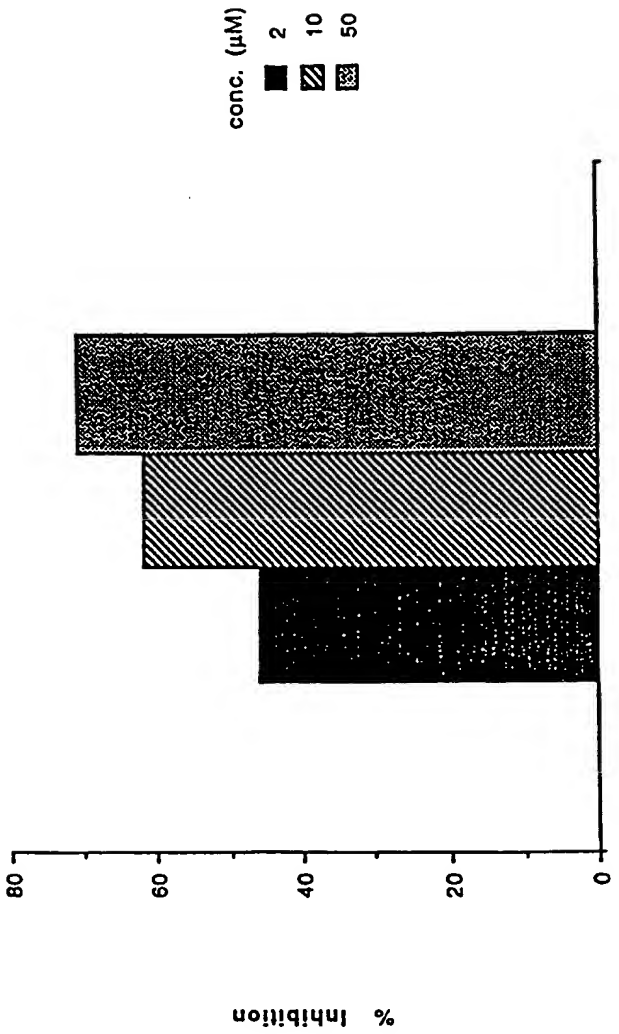

IL-1ß @ 20ng/ml
2507+
2507
+cntrl
No 2507
No IL-1ß
BCECF Fluorescence
6000
5000
4000
3000
2000
1000
0
.01
.1
1
10
100
Concentration (uM)

FIGURE 12



2507

FIGURE 13

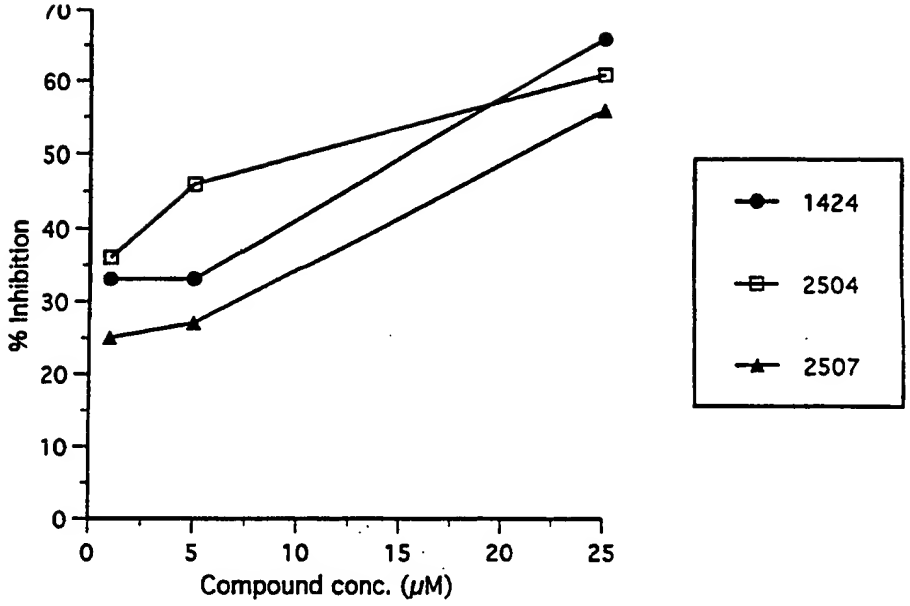

70
60
50
40
30
20
10
0
% Inhibition
0
5
10
15
20
25
Compound conc. (μM)
1424
2504
2507

FIGURE 14

INTERNATIONAL SEARCH REPORT

Int .tional application No.
PCT/US94/04007

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :544/271, 273, 309, 310; 546/219; 548/480; 514/263
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/271, 273, 309, 310; 546/219; 548/480; 514/263

Documentation searched other than minimum documentation to the extent tha' such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SYNTHESIS, No. 1, issued January 1993, Muller et al., "General Synthesis and Properties of 1-Monosubstituted Xanthines" pages 125-128. See compound 4g and its synthesis on page 126.	23, 26
X	Biochem. Pharmacology, Vol. 28, issued 1979, Croce et al, "Inhibition of alkaline Phosphatase by Substituted Xanthines" pages 1227-1231. See page 1229, compound 10.	23, 26
Y	J. Pharm. Pharmacology Vol. 41, issued 1989, Segura et al., "The Influence of Structure on the accumulation of Caffeine induced by methyl Xanthine Derivatives", pages 129-131. See page 129, compound K.	23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 JUNE 1994	Date of mailing of the international search report AUG 02 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARK L. BERCH jd Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/US94/04007

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,883,801 (Nathanson) 28 November 1989, See col. 13, species 22.	23
X	J. Heterocyclic Chemistry, Vol. 28, issued Aug-Sept. 1991, Ganjian et al., "Synthesis and Cytotoxic Activity of 2-Dialkylamino-alkyl-1,3-dihydropyrrolo[3,4-c]quinoline-1,3-dione s and 6-(2-Dimethylaminoethyl)-1H-dibenz[c,e]azepine-5,7-dione". Pages 1173-1175. See 9a on p. 1175.	23, 26
Y, P	J. Med. Chem. Vol. 35, Issued 1993, Muller et al. "Synthesis of Paraxanthine Analogs (1,7-Disubstituted Xanthines) and Other Xanthines Unsubstituted at the 3-Position: Structure-Activity Relationships at Adenosine Receptors". pages 3341-3349. See page 3344, species 47.	23
X	J. Org. Chem. Vol. 25, Issued April 1961, Nakano et al, "Structure of Julocrotine", pages 1184-1191. See page 1191.	23, 26
Y	J. Medicinal Chem. Volume 15, No. 5 issued 1972, Senda et al, "Pyrimidine Derivatives and Related Compounds 15. Synthesis and Analgetic and Antiinflammatory activities of 1,3-substituted 5-Amino-6-methyluracil Derivatives", pages 471-476. See pages 472-473, compounds 28 and 60.	23, 26

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US94/04007

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-22, 24-30
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US94/04007

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C07D 473/06, 473/10, 405/06, 239/54, 211/88, 209/48; A61K 31/52, 31/445, 31/40, 31/505

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

Claims 1-22 and 24-30 are not fully searched because they are so broad that their full scope can not be meaningfully searched. Only claim 23 could be meaningfully searched. As a result, claims 1-22 and 24-30 are examined only to the extent that they embrace the species of claim 23.

The compounds of claim 1 have a "core moiety" to which is attached 1-3 R groups. The core moiety is "cylic or non-cyclic", which covers everything. Even most dependent claims such as claim 12 define one of those choices, but do not require it. Thus, claim 12 defines a vast panapoly of non-cyclic cores, too broad to be searched, but even claim 12 does not require that the core be non-cyclic. Claim 19 does not narrow the core to three choices. However, in this and all other claims, R is too broad to be effectively searched, for essentially the same reason. Thus, even claims such as claim 10 do not require that R be "cyclic or heterocyclic", since claim 10 permits R to still be a group of formula I.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I Claims 1-29, compounds with xanthine core, plus use for acute inflammation. Classified in 544/271, 273; 514/263.
Group II Claims 1-29, compounds with phthalimide core, 548/480.
Group II Claims 1-29, compounds with glutarimide core, 546/219.
Group IV Claims 1-29, compounds with uracil core, 544/309, 310.
Group V Use for AIDS or ARC, 514/various.
Group VI Use for alcoholic hepatitis, 514/various.
Group VII Use for allergies due to degranulation of mast cells and basophils, 514/various.
Group VIII Use for angiogenesis, 514/various.
Group IX Use for asthma, 514/various.
Group X Use for atherosclerosis, 514/various.
Group XI Use for autoimmune thyroiditis, 514/various.
Group XII Use for coronary artery disease, 514/various.
Group XIII Use for glomerula nephritis, 514/various.
Group XIV Use for hairloss or baldness, 514/various.
Group XV Use for HIV-associated dementia, 514/various.
Group XVI Use for IBD, 514/various.
Group XVII Use for insulin dependent diabetes mellitus, 514/various.
Group XVIII Use for lupus, 514/various.
Group XIX Use for malignancies, 514/various.
Group XX Use for MS, 514/various.
Group XXI Use for myelogenous leukemia, 514/various.
Group XXII Use for organ response to cytotoxic therapy, 514/various.
Group XXIII Use for hematopoietic response to cytotoxic therapy, 514/various.
Group XXIV Use for osteoarthritic, 514/various.
Group XXV Use for osteoporosis, 514/various.
Group XXVI Use for peridontal disease, 514/various.
Group XXVII Use for premature labor secondary to uterine infection, 514/various.
Group XXVIII Use for psoriasis, 514/various.
Group XXIX Use for restenosis, 514/various.
Group XXX Use for rheumatoid arthritis, 514/various.
Group XXXI Use for sleep disorders, 514/various.
Group XXXII Use for septic shock or sepsis syndrome, 514/various.
Group XXXIII Use for scleroderma, 514/various.
Group XXXIV Use for stroke, 514/various.
Group XXXV Use for transplant rejection, 514/various.

The compound groups I-II-III-IV are clearly structurally distinct as seen by their markedly different cores. Group I is

distinctive in having 2 heterocyclic rings; Group II is distinctive in having a 6 membered ring with only one heteroatom; Group II is distinctive in having a five membered ring with the heteroatom, and in having a fused benzoring. The differing utilities are distinct because they involve different diseases, different types of origins, and different parts of the body. For example, some arise as a result of bacteria (sepsis) some from a virus (AIDS) and some not from an infectious agent (coronary artery disease). They involve different parts of the body, e.g.brain (stroke), liver (alcoholic hepatitis), intestines (IBD), skin (psoriasis), and others. Some are categories (malignancies) and some are considered extremely difficult or impossible to treat (HIV dementia, sepsis, CF), and others are more amenable (asthma).

Applicants should thus note that the search is done in the claim 23 species, their preparation, and use for chronic inflammatory disease.